LIVES IN MOLECULAR BIOLOGY

John Scales Avery

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INTRODUCTION[1]

Human history as cultural history

We need to reform our teaching of history so that the emphasis will be placed on the gradual growth of human culture and knowledge, a growth to which all nations and ethnic groups have contributed.

This book is part of a series on cultural history. Here is a list of the other books in the series that have, until now, been completed:

- Lives of Some Great Dramatists
- Lives in the Ancient World
- Lives in the Middle Ages
- Lives in the Renaissance
- Lives in the 17th Century
- Lives in the 18th Century
- Lives in the 19th Century
- Lives in the 20th century
- Lives in Biology
- Lives of Some Great Novelists
- Lives in Mathematics
- Lives in Exploration
- Lives in Education
- Lives in Poetry
- Lives in Painting
- Lives in Engineering
- Lives in Astronomy
- Lives in Chemistry
- Lives in Medicine
- Lives in Ecology
- Lives in Physics
- Lives in Economics
- Lives in the Peace Movement

[1]This book makes much use of my previously-published book chapters, but a considerable amount of new material has also been added.

The pdf files of these books may be downloaded and circulated free of charge from the following web addresses:

https://www.johnavery.info/

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Chapter 1

EHRLICH, MECHNIKOV AND JERNE

1.1 The language of molecular complementarity

In living (and even non-living) systems, signals can be written and read at the molecular level. The language of molecular signals is a language of complementarity. The first scientist to call attention to complementarity and pattern recognition at the molecular level was Paul Ehrlich, who was born in 1854 in Upper Silesia (now a part of Poland). Ehrlich was not an especially good student, but his originality attracted the attention of his teacher, Professor Waldeyer, under whom he studied chemistry at the University of Strasbourg. Waldeyer encouraged him to do independent experiments with the newly-discovered aniline dyes; and on his own initiative, Ehrlich began to use these dyes to stain bacteria. He was still staining cells with aniline dyes a few years later (by this time he had become a medical student at the University of Breslau) when the great bacteriologist Robert Koch visited the laboratory. "This is young Ehrlich, who is very good at staining, but will never pass his examinations", Koch was told. Nevertheless, Ehrlich did pass his examinations, and he went on to become a doctor of medicine at the University of Leipzig at the age of 24. His doctoral thesis dealt with the specificity of the aniline dyes: Each dye stained a special class of cell and left all other cells unstained.

Paul Ehrlich had discovered what might be called "the language of molecular complementarity": He had noticed that each of his aniline dyes stained only a particular type of tissue or a particular species of bacteria. For example, when he injected one of his blue dyes into the ear of a rabbit, he found to his astonishment that the dye molecules attached themselves selectively to the nerve endings. Similarly, each of the three types of phagocytes could be stained with its own particular dye, which left the other two kinds unstained[1].

[1] The specificity which Ehrlich observed in his staining studies made him hope that it might be possible to find chemicals which would attach themselves selectively to pathogenic bacteria in the blood stream and kill the bacteria without harming normal body cells. He later discovered safe cures for both sleeping sickness and syphilis, thus becoming the father of chemotherapy in medicine. He had already received the Nobel Prize for his studies of the mechanism of immunity, but after his discovery of a cure for syphilis, a

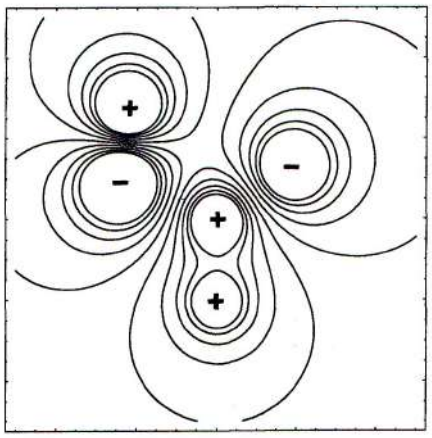

Figure 1.1: **This figure shows the excess charges and the resulting electrostatic potential on a molecule of formic acid, HCOOH. The two oxygens in the carboxyl group are negatively charged, while the carbon and the two hydrogens have positive excess charges. Molecular recognition involves not only steric complementarity, but also complementarity of charge patterns.**

Ehrlich believed that this specificity came about because the side chains on his dye molecules contained groupings of atoms which were complementary to groups of atoms on the surfaces of the cells or bacteria which they selectively stained. In other words, he believed that biological specificity results from a sort of lock and key mechanism: He visualized a dye molecule as moving about in solution until it finds a binding site which exactly fits the pattern of atoms in one of its side chains. Modern research has completely confirmed this picture, with the added insight that we now know that the complementarity of the "lock" and "key" is electrostatic as well as spatial.

Two molecules in a biological system may fit together because the contours of one are complementary to the contours of the other. This is how Paul Ehrlich visualized the fit - a spatial (steric) complementarity, like that of a lock and key. However, we now know that for maximum affinity, the patterns of excess charges on the surfaces of the two molecules must also be complementary. Regions of positive excess charge on the surface of one molecule must fit closely with regions of negative excess charge on the other if the two are to bind maximally. Thus the language of molecules is not only a language of contours, but also a language of charge distributions.

1.2 Paul Ehrlich, the father of chemotherapy

The first real understanding of the mechanism of the immune system was due to the work of Paul Ehrlich and Ilya Mechnikov, and in 1908 they shared a Nobel Prize for this work. Paul

street in Frankfurt was named after him!

Ehrlich can be said to be the discoverer of biological specificity. As a young medical student at the University of Strasbourg, he was fortunate to work under the distinguished chemist Heinrich von Waldeyer, who took a great interest in Ehrlich. Stimulated by Waldeyer, Ehrlich began to do experiments in which he prepared thin slices of various tissues for microscopic examination by staining them with the newly discovered aniline dyes. During the last half of the 19th century, there was a great deal of interest in histological staining. It was during this period that Walther Flemming in Germany discovered chromosomes by staining them with special dyes, and Christian Gram in Denmark showed that bacteria can be classified into two types by staining methods. (We now call these two types "gram positive" and "gram negative"). During this same period, and while he was still a student, Paul Ehrlich made the important discovery that mammalian blood contains three different types of white cells which can be distinguished by staining.

Ehrlich's early work on staining made him famous, and it also gave him a set of theories which led him to his great discoveries in immunology and chemotherapy. According to Ehrlich's ideas, the color of the aniline dyes is due to the aniline ring. However, dyes used commercially must also adhere to fabrics, and this adherence, according to Ehrlich, is due to the specific structure of the side chains. If the pattern of atoms on a side chain is complementary to the pattern of atoms on the binding site, the dye will adhere, but otherwise not. Thus there is a "lock and key" mechanism, and for this reason dyes with specific side chains stain specific types of tissue.

In one of his experiments, Paul Ehrlich injected methylene blue into the ear of a living rabbit, and found that it stained only the nerve endings of the rabbit. Since the rabbit seemed to be unharmed by the treatment, the experiment suggested to Ehrlich that it might be possible to find antibacterial substances which could be safely injected into the bloodstream of a patient suffering from an infectious disease. Ehrlich hoped to find substances which would adhere selectively to the bacteria, while leaving the tissues of the patient untouched.

With the help of a large laboratory especially constructed for him in Frankfurt, the center of the German dye industry, Ehrlich began to screen thousands of modified dyes and other compounds. In this way he discovered trypan red, a chemical treatment for sleeping sickness, and arsphenamine, a drug which would cure syphilis. Ehrlich thus became the father of modern chemotherapy. His success pointed the way to Gerhard Domagk, who discovered the sulphonamide drugs in the 1930s, and to Fleming, Waksman, Dubos and others, who discovered the antibiotics.

Ehrlich believed that in the operation of the immune system, the body produces molecules which have a pattern of atoms complementary to patterns (antigens) on invading bacteria, and that these molecules (antibodies) in the blood stream kill the bacteria by adhering to them.

Figure 1.2: **Paul Ehrlich (1854-1915). By the time that he developed a drug that could cure syphilis, he had already received the Nobel Prize for Physiology or Medicine, but to further honor Ehrlich, a street in Frankfurt was named after him**

Figure 1.3: **Dr. Paul Ehrlich and his assistant Dr. Sahachiro Hata. They worked together to find cures for many diseases.**

Figure 1.4: **A West German postage stamp (1954) commemorating Paul Ehrlich and Emil von Behring, who worked together at Robert Koch's suggestion, producing a drug that could cure diptheria.**

1.3 Mechnikov

Meanwhile, the Russian naturalist Ilya Mechnikov discovered another mechanism by which the immune system operates. While on vacation in Sicily, Mechnikov was studying the digestive process in starfish larvae. In order to do this, he introduced some particles of carmine into the larvae. The starfish larvae were completely transparent, and thus Mechnikov could look through his microscope and see what happened to the particles. He saw that they were enveloped and apparently digested by wandering amoebalike cells inside the starfish larvae. As he watched this process, it suddenly occurred to Mechnikov that our white cells might similarly envelop and digest bacteria, thus protecting us from infection. Describing this discovery, Mechnikov wrote in his diary: "I suddenly became a pathologist! Feeling that there was in this idea something of surpassing interest, I became so excited that I began striding up and down the room, and even went to the seashore to collect my thoughts."

Mechnikov later named the white cells "phagocytes" (which means "eating cells"). He was able to show experimentally that phagocytosis (i.e., the envelop- ment and digestion of bacteria by phagocytes) is an important mechanism in immunity.

Metchnikov's ideas were not immediately accepted. Wikipedia states that "His theory, that certain white blood cells could engulf and destroy harmful bodies such as bacteria, met with scepticism from leading specialists including Louis Pasteur, Behring and others. At the time, most bacteriologists believed that white blood cells ingested pathogens and then spread them further through the body. His major supporter was Rudolf Virchow, who published his research in his *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin* (now called the Virchows Archiv). His discovery of these phagocytes ultimately won him the Nobel Prize in 1908."

For a number of years, there were bitter arguments between those who thought that the immune system operates through phagocytosis, and those who thought that it operates through antibodies. Finally it was found that both mechanisms play a role. In phagocytosis, the bacterium will not be ingested by the phagocyte unless it is first studded with antibodies. Thus both Mechnikov and Ehrlich were proved to be right.

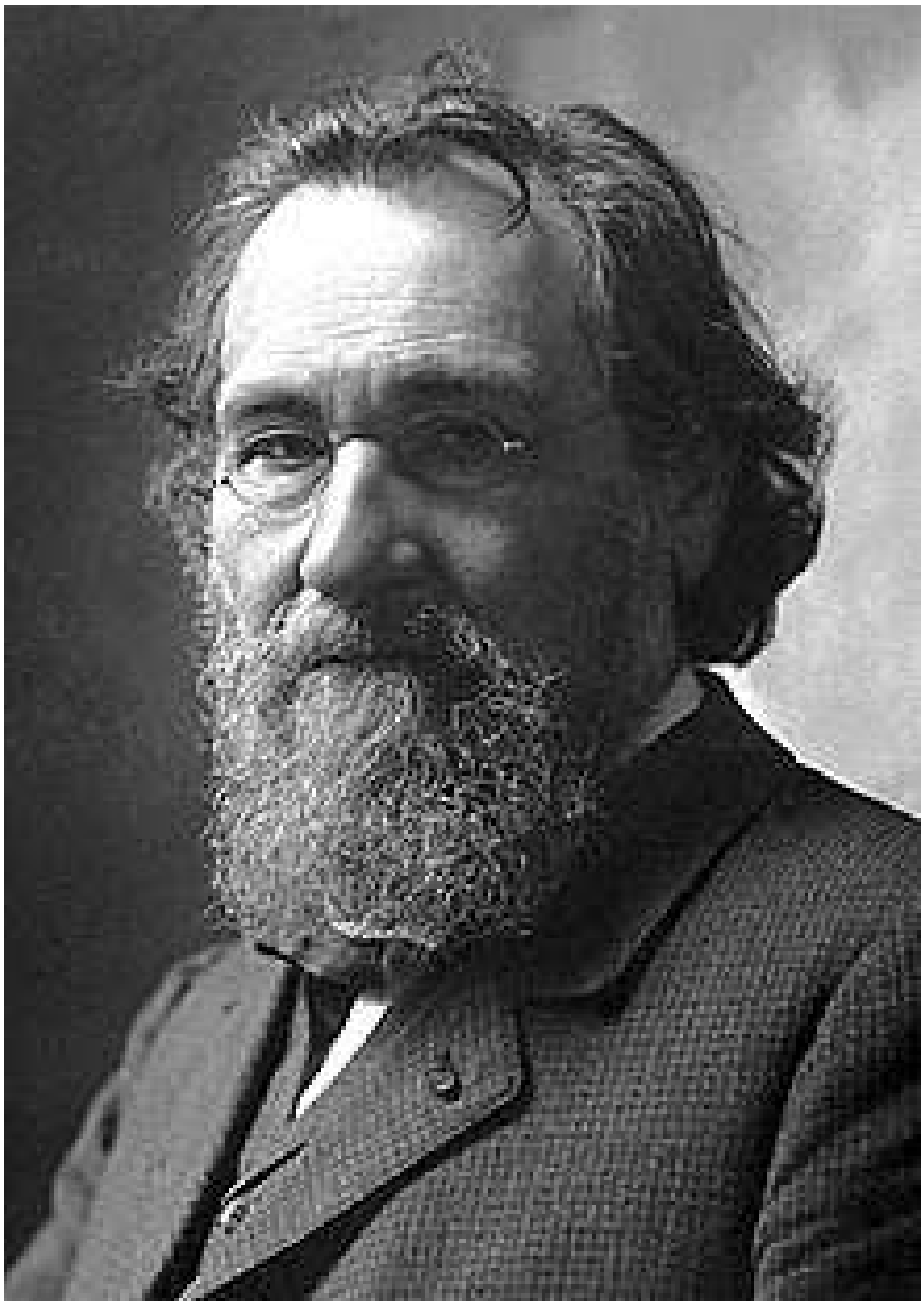

Figure 1.5: **Ilya Mechnikov (1845-1916), sometimes spelled Élie Metchnikoff. He shared the 1908 Nobel Prize in Physiology or Medicine with Paul Ehrlich. Mechnikov has been called "the father of immunology" because of his discovery of phagocytosis.**

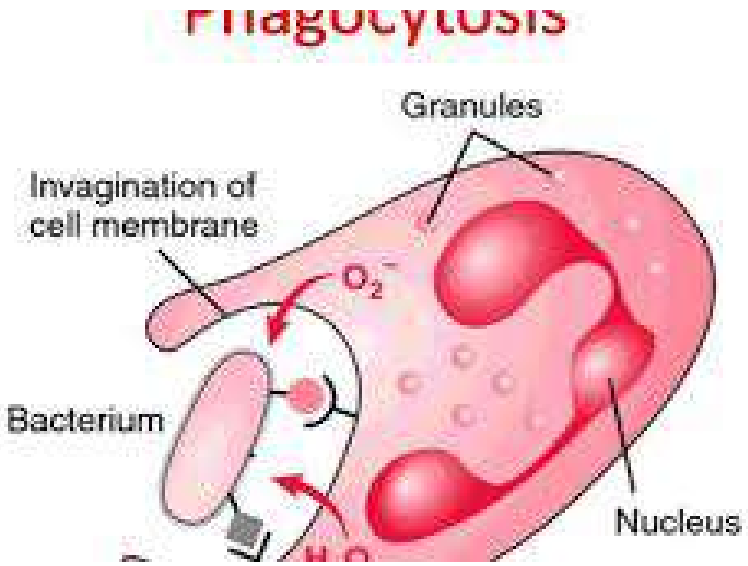

Figure 1.6: **Phagocytosis: A lymphocyte "eats" a bacterium, but only if it is coated with the right antigens.**

1.4 Burnet, Jerne and the clonal theory of immunity

As everyone knows, recovery from an infectious disease involves a response of our immune systems. Recovery occurs after the immune system had had some time to respond, and a recovered patient generally has some immunity to the disease.

During the 20th century, there were conflicting ideas about how and why this process occurs. One of these theories was proposed by Linus Pauling, who thought that an antigen on the surface of a bacteria or virus provides a template, and that the immune system uses this template to produce the specific antibodies needed to combat the disease. However, experimental evidence accumulated showing Pauling's template theory to be wrong and supporting the clonal theory of immunity proposed by Sir Frank Macfarlane Burnet and Niels Kai Jerne.

According to the clonal theory of immunity, there are extremely many strains of lymphocytes, each of which produces a specific single antibody. Populations of all these many strains are always present in small numbers. When a patient becomes ill with an infection, the antigens of the ingesting bacteria or virus stimulate one specific strain of lymphocyte to reproduce itself in large numbers, i.e. to become a clone. This large population produces exactly the right antibodies needed to combat the disease, and the large population remains after recovery, conferring continued immunity.

In order for the immune system not to attack the cells of our own bodies, a learning process must take place, early in our lives, in which the difference between self and non-self is established, and the lymphocyte strains that attack self are suppressed. Jerne postulated (correctly) that this learnubg process takes place in the thymus gland, which is very large in infants, and much smaller in adults.

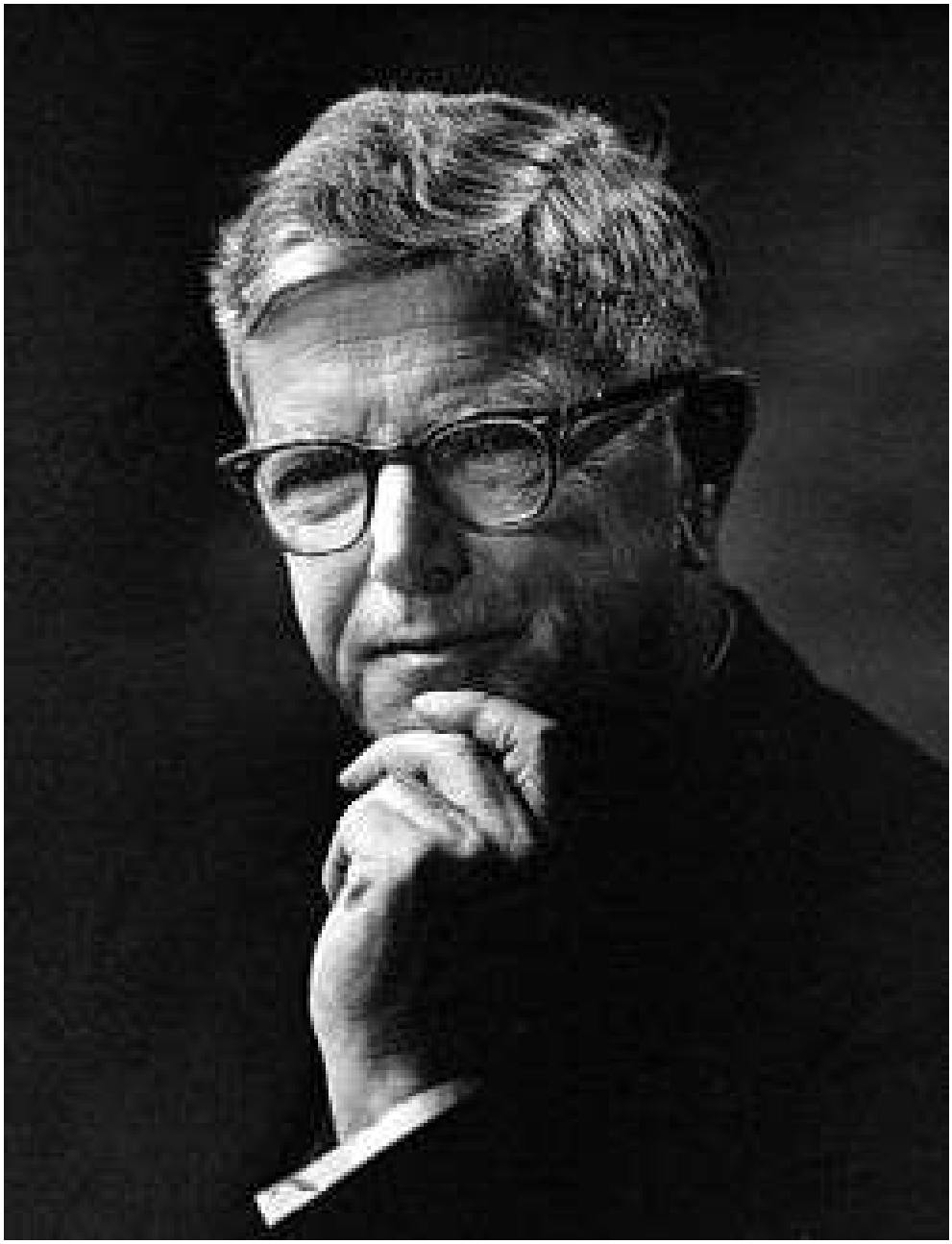

Figure 1.7: **Sir Frank Macfarlane Burnet (1899-1995). Both he and Niels Kai Jerne proposed the clonal theory of immunity.**

Figure 1.8: **The Danish immunologist Niels Kai Jerne (1911-1994). He shared the 1984 Nobel Prize for Physiology or Medicine with Georges Köhler and César Milstein "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies".**

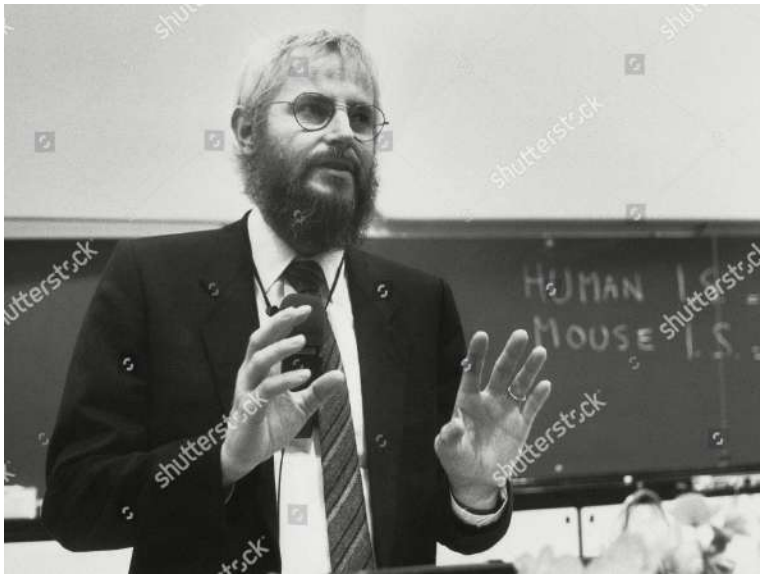

Figure 1.9: **Georges Köhler (1046-1995).**

Figure 1.10: **César Milstein (1927-2002).**

1.5 Köhler, Milstein and monoclonal antibodies

Once the clonal theory of immunity became established, the way seemed open to clone in vitro B lymphocytes of a predetermined specificity. However, such clone cannot be made to live forever because like all other cells, except cancer cells, they are subject to "programed cell death". To overcome this difficulty, Georges Köhler and César Milstein found a way to give the desired lymphocytes immortality by fusing them with myoloma cells, thus producing clones that could be cultured indefinitely.

The Wikipedia article on Monoclonal Antibodies states that "In the 1970s, the B-cell cancer multiple myoloma was known. It was understood that these cancerous B-cells all produce a single type of antibody (a paraprotein). This was used to study the structure of antibodies, but it was not yet possible to produce identical antibodies specific to a given antigen.

"In 1975, Georges Köhler and César Milstein succeeded in making fusions of myeloma cell lines with B cells to create hybridomas that could produce antibodies, specific to known antigens and that were immortalized. They and Niels Kaj Jerne shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery.

"In 1988, Greg Winter and his team pioneered the techniques to humanize monoclonal antibodies, eliminating the reactions that many monoclonal antibodies caused in some patients.

"In 2018, James P. Allison and Tasuku Honjo received the Nobel Prize in Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation, using monoclonal antibodies that prevent inhibitory linkages."

Suggestions for further reading

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Chapter 2

CRICK AND WATSON

2.1 The structure of proteins

X-ray crystallography

In England, J.D. Bernal and Dorothy Crowfoot Hodgkin pioneered the application of X-ray diffraction methods to the study of complex biological molecules. In 1949, Hodgkin determined the structure of penicillin; and in 1955, she followed this with the structure of vitamin B12. In 1960, Max Perutz and John C. Kendrew obtained the structures of the blood proteins myoglobin and hemoglobin. This was an impressive achievement for the Cambridge crystallographers, since the hemoglobin molecule contains roughly 12,000 atoms.

The structure obtained by Perutz and Kendrew showed that hemoglobin is a long chain of amino acids, folded into a globular shape, like a small, crumpled ball of yarn. They found that the amino acids with an affinity for water were on the outside of the globular molecule; while the amino acids for which contact with water was energetically unfavorable were hidden on the inside. Perutz and Kendrew deduced that the conformation of the protein - the way in which the chain of amino acids folded into a 3-dimensional structure - was determined by the sequence of amino acids in the chain.

In 1966, D.C. Phillips and his co-workers at the Royal Institution in London found the crystallographic structure of the enzyme lysozyme (an egg-white protein which breaks down the cell walls of certain bacteria). Again, the structure showed a long chain of amino acids, folded into a roughly globular shape. The amino acids with hydrophilic groups were on the outside, in contact with water, while those with hydrophobic groups were on the inside. The structure of lysozyme exhibited clearly an active site, where sugar molecules of bacterial cell walls were drawn into a mouth-like opening and stressed by electrostatic forces, so that bonds between the sugars could easily be broken.

Meanwhile, at Cambridge University, Frederick Sanger developed methods for finding the exact sequence of amino acids in a protein chain. In 1945, he discovered a compound (2,4-dinitrofluorobenzene) which attaches itself preferentially to one end of a chain of amino acids. Sanger then broke down the chain into individual amino acids, and determined which

Figure 2.1: **Dorothy Crowfoot Hodgkin (1910-1994). She and her mentor J.D Bernal were a great pioneers in the application of X-ray crystallogrography to determination of the structure of biological molecules, such as proteins. She was awarded the Nobel Prize in Chemistry in 1964.**

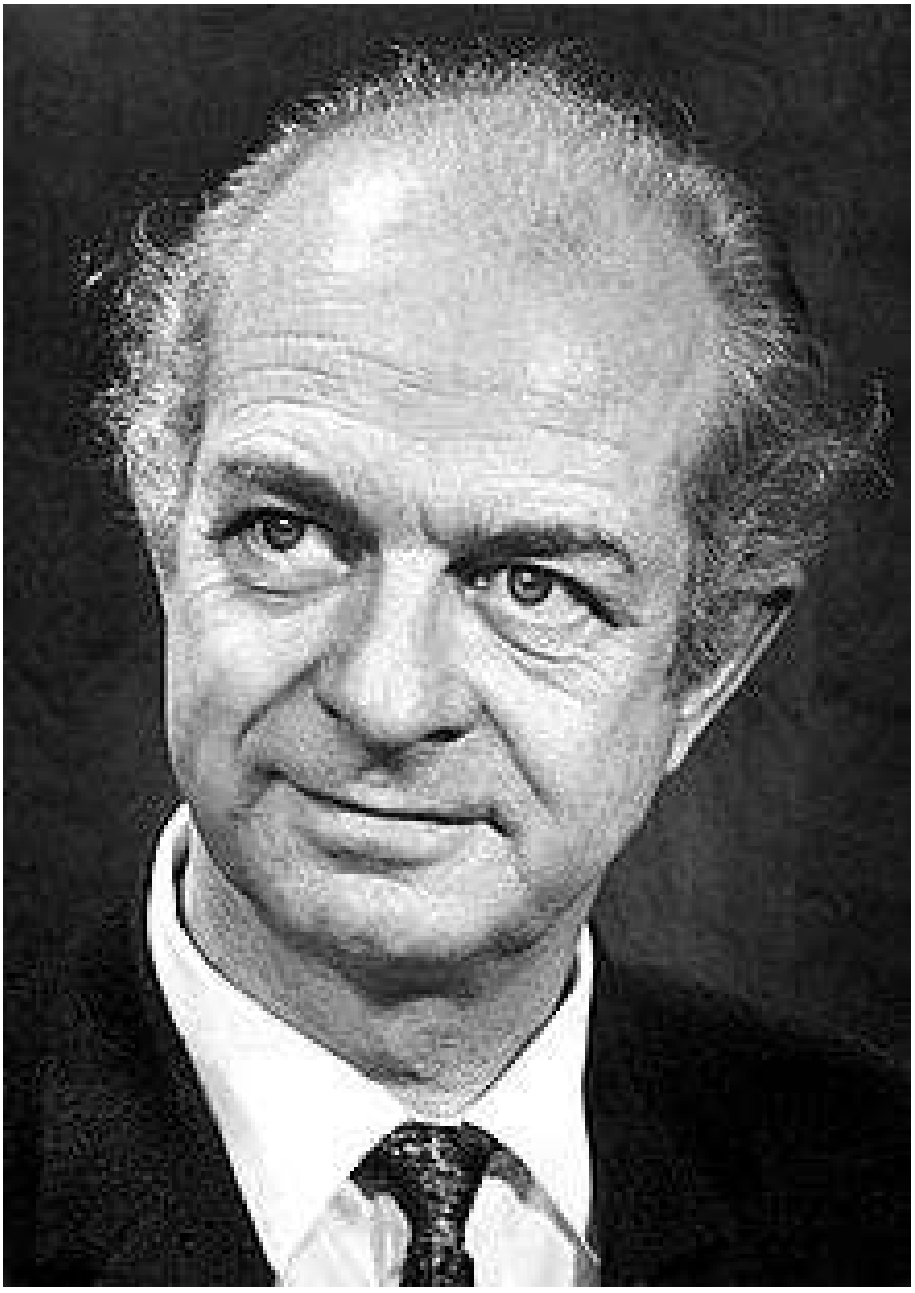

Figure 2.2: **Linus Pauling (1901-1994). The New Scientist called him one of the 20 most important scientists in history. He was awarded the Nobel Prize in Chemistry in 1954 and the Nobel Peace Prize in 1962.**

Figure 2.3: **Frederick Sanger (1918-2013) was one of the only two people in history have won two Nobel Prizes in the same field, in his case Chemistry. He won the first on 1958 for his work on the structure of proteins, and the second in 1980 for his method for determining the base sequences of nucleic acids.**

of them was connected to his reagent. By applying this procedure many times to fragments of larger chains, Sanger was able to deduce the sequence of amino acids in complex proteins. In 1953, he published the sequence of insulin. This led, in 1964, to the synthesis of insulin.

Linus Pauling also contributed importantly to our understanding of the structure of proteins. Wikipedia says of his work: "Pauling was one of the founders of the fields of quantum chemistry and molecular biology. His contributions to the theory of the chemical bond include the concept of orbital hybridisation and the first accurate scale of electronegativities of the elements. Pauling also worked on the structures of biological molecules, and showed the importance of the alpha helix and beta sheet in protein secondary structure. Pauling's approach combined methods and results from X-ray crystallography, molecular model building, and quantum chemistry. His discoveries inspired the work of James Watson, Francis Crick, and Rosalind Franklin on the structure of DNA, which in turn made it possible for geneticists to crack the DNA code of all organisms."

The biological role and structure of proteins which began to emerge was as follows: A mammalian cell produces roughly 10,000 different proteins. All enzymes are proteins; and the majority of proteins are enzymes - that is, they catalyze reactions involving other biological molecules. All proteins are built from chainlike polymers, whose monomeric sub-units are the following twenty amino acids: glycine, aniline, valine, isoleucine, leucine, serine, threonine, proline, aspartic acid, glutamic acid, lysine, arginine, asparagine, glutamine, cysteine, methionine, tryptophan, phenylalanine, tyrosine and histidine. These individual amino acid monomers may be connected together into a polymer (called a polypeptide) in any order - hence the great number of possibilities. In such a polypeptide, the backbone is a chain of carbon and nitrogen atoms showing the pattern ...-C-C-N-C-C-N-C-C-N-...and so on. The -C-C-N- repeating unit is common to all amino acids. Their individuality is derived from differences in the side groups which are attached to the universal -C-C-N- group.

Some proteins, like hemoglobin, contain metal atoms, which may be oxidized or reduced as the protein performs its biological function. Other proteins, like lysozyme, contain no metal atoms, but instead owe their biological activity to an active site on the surface of the protein molecule. In 1909, the English physician, Archibald Garrod, had proposed a one-gene-one-protein hypothesis. He believed that hereditary diseases are due to the absence of specific enzymes. According to Garrod's hypothesis, damage suffered by a gene results in the faulty synthesis of the corresponding enzyme, and loss of the enzyme ultimately results in the symptoms of the hereditary disease.

In the 1940's, Garrod's hypothesis was confirmed by experiments on the mold, Neurospora, performed at Stanford University by George Beadle and Edward Tatum. They demonstrated that mutant strains of the mold would grow normally, provided that specific extra nutrients were added to their diets. The need for these dietary supplements could in every case be traced to the lack of a specific enzyme in the mutant strains. Linus Pauling later extended these ideas to human genetics by showing that the hereditary disease, sickle-cell anemia, is due to a defect in the biosynthesis of hemoglobin.

2.2 What is Life?

What is Life? That was the title of a small book published by the physicist Erwin Schrödinger in 1944. Schrödinger (1887-1961) was born and educated in Austria. In 1926 he shared the Nobel Prize in Physics[1] for his contributions to quantum theory (wave mechanics). Schrödinger's famous wave equation is as fundamental to modern physics as Newton's equations of motion are to classical physics.

When the Nazis entered Austria in 1938, Schrödinger opposed them, at the risk of his life. To escape arrest, he crossed the Alps on foot, arriving in Italy with no possessions except his knapsack and the clothes which he was wearing. He traveled to England; and in 1940 he obtained a position in Ireland as Senior Professor at the Dublin Institute for Advanced Studies. There he gave a series of public lectures upon which his small book is based.

In his book, *What is Life?*, Schrödinger developed the idea that a gene is a very large information-containing molecule which might be compared to an aperiodic crystal. He also examined in detail the hypothesis (due to Max Delbrück) that X-ray induced mutations of the type studied by Hermann Muller can be thought of as photo-induced transitions from one isomeric conformation of the genetic molecule to another. Schrödinger's book has great historic importance, because Francis Crick (whose education was in physics) was one of the many people who became interested in biology as a result of reading it. Besides discussing what a gene might be in a way which excited the curiosity and enthusiasm of Crick, Schrödinger devoted a chapter to the relationship between entropy and life.

"What is that precious something contained in our food which keeps us from death? That is easily answered," Schrödinger wrote, "Every process, event, happening - call it what you will; in a word, everything that is going on in Nature means an increase of the entropy of the part of the world where it is going on. Thus a living organism continually increases its entropy - or, as you may say, produces positive entropy, which is death. It can only keep aloof from it, i.e. alive, by continually drawing from its environment negative entropy - which is something very positive as we shall immediately see. What an organism feeds upon is negative entropy. Or, to put it less paradoxically, the essential thing in metabolism is that the organism succeeds in freeing itself from all the entropy it cannot help producing while alive..."[2]

"Entropy, taken with a negative sign, is itself a measure of order. Thus the device by which an organism maintains itself stationary at a fairly high level of orderliness (= fairly low level of entropy) really consists in continually sucking orderliness from its environment. This conclusion is less paradoxical than it appears at first sight. Rather it could be blamed for triviality. Indeed, in the case of higher animals we know the kind of orderliness they feed upon well enough, viz. the extremely well-ordered state of matter state in more or less complicated organic compounds which serve them as foodstuffs. After utilizing it, they

[1] with P.A.M. Dirac

[2] The Hungarian-American biochemist Albert Szent-Györgyi, who won a Nobel prize for isolating vitamin C, and who was a pioneer of Bioenergetics, expressed the same idea in the following words: "We need energy to fight against entropy".

Figure 2.4: **The great Austrian physicist Erwin Schrödinger (1887-1961) was one of the principle founders of quantum theory. He fled from Austria over the mountains to Italy after the Nazis entered his country, and finally found refuge at the Institute for Advanced Studies in Ireland. It was there that he wrote his important book, "What is Life?". Reading Schrödinger's book, Francis Crick was inspired to look for the structure of DNA.**

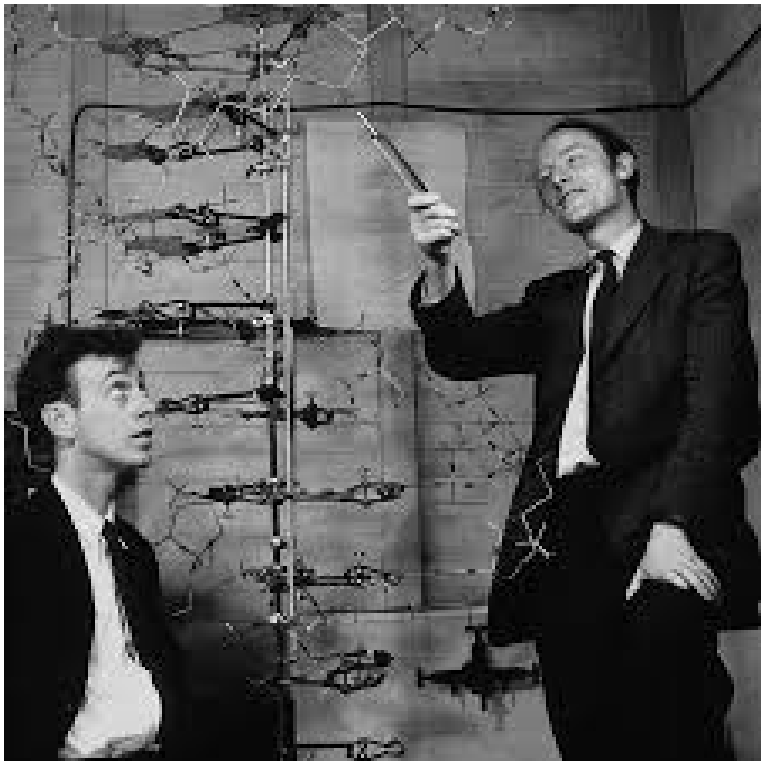

Figure 2.5: **Francis Crick (1916-2004) and James Dewey Watson (born 1928) at the Cavendish Laboratory with their model of DNA. After their discovery of the structure of DNA, it became clear that it was this molecule that carried genetic information between generations.**

return it in a very much degraded form - not entirely degraded, however, for plants can still make use of it. (These, of course, have their most powerful source of 'negative entropy' in the sunlight.)" At the end of the chapter, Schrödinger added a note in which he said that if he had been writing for physicists, he would have made use of the concept of free energy; but he judged that this concept might be difficult or confusing for a general audience.

All living organisms draw a supply of thermodynamic information from their environment, and they use it to "keep aloof" from the disorder which constantly threatens them. In the case of animals, the information-containing free energy comes in the form of food. In the case of green plants, it comes primarily from sunlight. The thermodynamic information thus gained by living organisms is used by them to create configurations of matter which are so complex and orderly that the chance that they could have arisen in a random way is infinitesimally small.

John von Neumann invented a thought experiment which illustrates the role which free energy plays in creating statistically unlikely configurations of matter. Von Neumann imagined a robot or automaton, made of wires, electrical motors, batteries, etc., constructed in such a way that when floating on a lake stocked with its component parts, it will reproduce itself. The important point about von Neumann's automaton is that it requires a source of

free energy (i.e., a source of energy from which work can be obtained) in order to function. We can imagine that the free energy comes from electric batteries which the automaton finds in its environment. (These are analogous to the food eaten by animals.) Alternatively we can imagine that the automaton is equipped with photocells, so that it can use sunlight as a source of free energy, but it is impossible to imagine the automaton reproducing itself without some energy source from which work can be obtained to drive its reproductive machinery. If it could be constructed, would von Neumann's automaton be alive? Few people would say yes. But if such a self-reproducing automaton could be constructed, it would have some of the properties which we associate with living organisms.

The autocatalysts which are believed to have participated in molecular evolution had some of the properties of life. They used "food" (i.e., energy-rich molecules in their environments) to reproduce themselves, and they evolved, following the principle of natural selection. The autocatalysts were certainly precursors of life, approaching the borderline between non-life and life.

Is a virus alive? We know, for example, that the tobacco mosaic virus can be taken to pieces. The proteins and RNA of which it is composed can be separated, purified, and stored in bottles on a laboratory shelf. At a much later date, the bottles containing the separate components of the virus can be taken down from the shelf and incubated together, with the result that the components assemble themselves in the correct way, guided by steric and electrostatic complementarity. New virus particles are formed by this process of autoassembly, and when placed on a tobacco leaf, the new particles are capable of reproducing themselves. In principle, the stage where the virus proteins and RNA are purified and placed in bottles could be taken one step further: The amino acid sequences of the proteins and the base sequence of the RNA could be determined and written down.

Later, using this information, the parts of the virus could be synthesized from amino acids and nucleotides. Would we then be creating life? Another question also presents itself: At a certain stage in the process just described, the virus seems to exist only in the form of information - the base sequence of the RNA and the amino acid sequence of the proteins. Can this information be thought of as the idea of the virus in the Platonic sense? (Pythagoras would have called it the "soul" of the virus.) Is a computer virus alive? Certainly it is not so much alive as a tobacco mosaic virus. But a computer virus can use thermodynamic information (supplied by an electric current) to reproduce itself, and it has a complicated structure, containing much cybernetic information.

Under certain circumstances, many bacteria form spores, which do not metabolize, and which are able to exist without nourishment for very long periods - in fact for millions of years. When placed in a medium containing nutrients, the spores can grow into actively reproducing bacteria. There are examples of bacterial spores existing in a dormant state for many millions of years, after which they have been revived into living bacteria. Is a dormant bacterial spore alive?

Clearly there are many borderline cases between non-life and life; and Aristotle seems to have been right when he said, "Nature proceeds little by little from lifeless things to animal life, so that it is impossible to determine either the exact line of demarcation, or on which side of the line an intermediate form should lie." However, one theme seems to characterize

life: It is able to convert the thermodynamic information contained in food or in sunlight into complex and statistically unlikely configurations of matter. A flood of information-containing free energy reaches the earth's biosphere in the form of sunlight. Passing through the metabolic pathways of living organisms, this information keeps the organisms far away from thermodynamic equilibrium ("which is death"). As the thermodynamic information flows through the biosphere, much of it is degraded into heat, but part is converted into cybernetic information and preserved in the intricate structures which are characteristic of life. The principle of natural selection ensures that as this happens, the configurations of matter in living organisms constantly increase in complexity, refinement and statistical improbability. This is the process which we call evolution, or in the case of human society, progress.

2.3 The structure of DNA

Until 1944, most scientists had guessed that the genetic message was carried by the proteins of the chromosome. In 1944, however, O.T. Avery and his co-workers at the laboratory of the Rockefeller Institute in New York performed a critical experiment, which proved that the material which carries genetic information is not protein, but deoxyribonucleic acid (DNA) - a giant chainlike molecule which had been isolated from cell nuclei by the Swiss chemist, Friedrich Miescher.

Avery had been studying two different strains of pneumococci, the bacteria which cause pneumonia. One of these strains, the S-type, had a smooth coat, while the other strain, the R-type, lacked an enzyme needed for the manufacture of a smooth carbohydrate coat. Hence, R-type pneumococci had a rough appearance under the microscope. Avery and his co-workers were able to show that an extract from heat-killed S-type pneumococci could convert the living R-type species permanently into S-type; and they also showed that this extract consisted of pure DNA.

In 1947, the Austrian-American biochemist, Erwin Chargaff, began to study the long, chainlike DNA molecules. It had already been shown by Levine and Todd that chains of DNA are built up of four bases: adenine (A), thymine (T), guanine (G) and cytosine (C), held together by a sugar-phosphate backbone. Chargaff discovered that in DNA from the nuclei of living cells, the amount of A always equals the amount of T; and the amount of G always equals the amount of C.

When Chargaff made this discovery, neither he nor anyone else understood its meaning. However, in 1953, the mystery was completely solved by Rosalind Franklin and Maurice Wilkins at Kings College, London, together with James Watson and Francis Crick at Cambridge University. By means of X-ray diffraction techniques, Wilkins and Franklin obtained crystallographic information about the structure of DNA. Using this information, together with Linus Pauling's model-building methods, Crick and Watson proposed a detailed structure for the giant DNA molecule.

The discovery of the molecular structure of DNA was an event of enormous importance for genetics, and for biology in general. The structure was a revelation! The giant, helical

Figure 2.6: **Sir Francis Crick (1916-2004). Besides being half of the team that determined the correct structure of DNA, he made many other extremely important contributions to molecular biology and neuroscience. He contributed importantly to the solution of the genetic code, and is known for his "central dogma": Information flows from DNA to RNA, and never backward. RNA codes the synthesis of proteins, and enzymes, which are proteins, catalyze the synthesis of smaller molecules.**

Figure 2.7: **James Dewey Watson (born in 1928) Crick's partner in solving the DNA structure. After serving for 35 years as Director and later President of the Cold Springs Harbor Laboratory and greatly expanding it facilities, he joined the US National Institutes of Health, where he has been the driving force behind the Human Genome Project.**

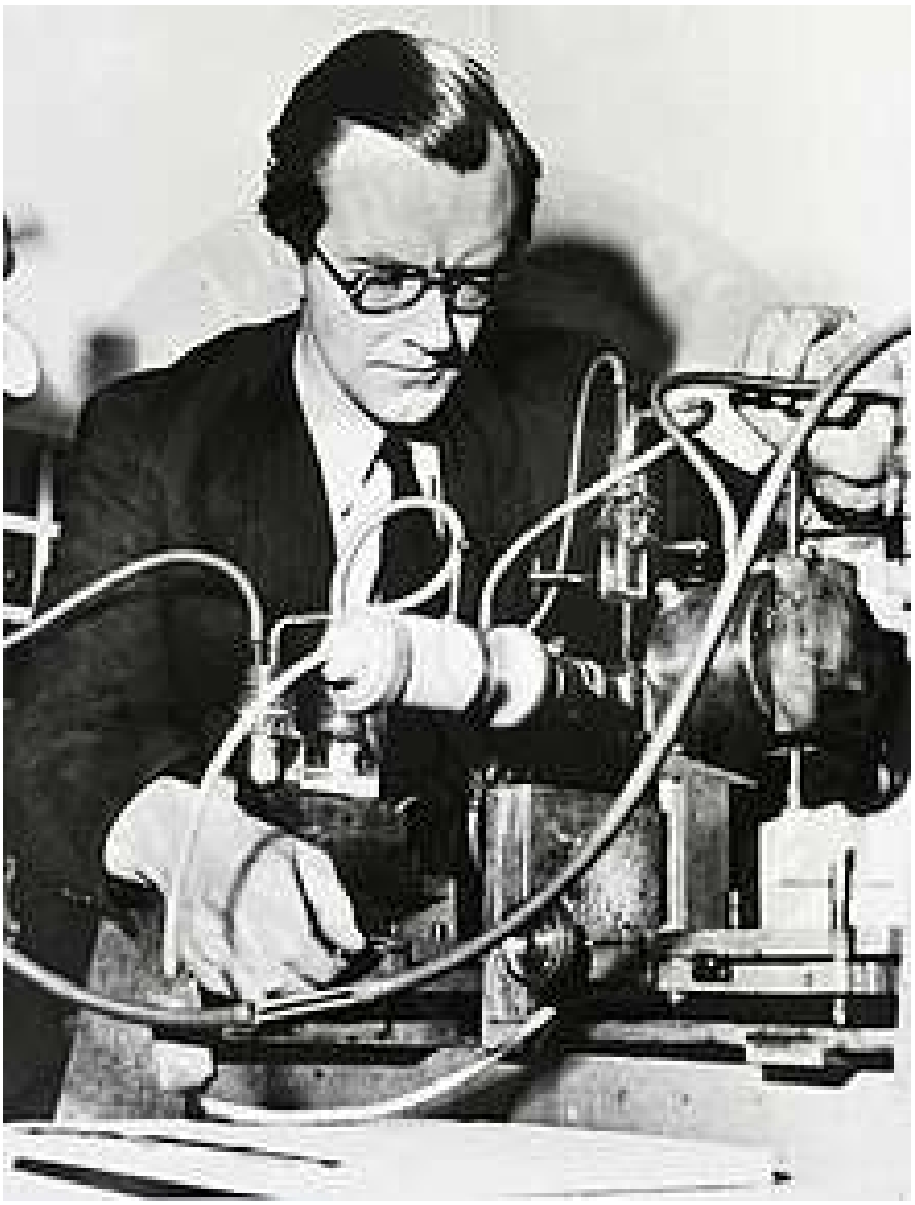

Figure 2.8: **Maurice Wilkins (1916-2004). He applied to DNA the X-ray diffraction methods pioneered by Dorothy Hodgkin. It was his work, and that of Rosalind Franklin, together with Linus Pauling's model-building methods, that enabled Crick and Watson to correctly solve the structure of DNA. He shared the 1962 Nobel Prize in Physiology or Medicine with them.**

Figure 2.9: **Rosalind Franklin (1920-1958). It was one of her high-quality diffraction photographs, taken in Maurice Wilkins' laboratory, that proved to be critical for the DNA structure. She might have shared the Nobel Prize with Wilkins, Crick and Watson, but before this could be considered by the committee, she died of overian cancer.**

Figure 2.10: **Oswald Theodore Avery (1877-1955). Together with his team at the Rockefeller University Hospital in New York City, he proved experimentally that DNA is the molecule that carries genetic information between generations.**

Figure 2.11: **The Austro-Hungarian biochemist Erwin Chargaff (1905-2002) found experimentally that in DNA from the nuclei of living cells, the amount of adenine always equals the amount of thiamine; and the amount of guanine always equals the amount of cytosine, but at the time of his discovery, neither he nor anyone else, understood the meaning of this rule.**

DNA molecule was like a twisted ladder: Two long, twisted sugar-phosphate backbones formed the outside of the ladder, while the rungs were formed by the base pairs, A, T, G and C. The base adenine (A) could only be paired with thymine (T), while guanine (G) fit only with cytosine (C). Each base pair was weakly joined in the center by hydrogen bonds - in other words, there was a weak point in the center of each rung of the ladder - but the bases were strongly attached to the sugar-phosphate backbone. In their 1953 paper, Crick and Watson wrote:

"It has not escaped our notice that the specific pairing we have postulated suggests a possible copying mechanism for genetic material". Indeed, a sudden blaze of understanding illuminated the inner workings of heredity, and of life itself.

If the weak hydrogen bonds in the center of each rung were broken, the ladderlike DNA macromolecule could split down the center and divide into two single strands. Each single strand would then become a template for the formation of a new double-stranded molecule.

Because of the specific pairing of the bases in the Watson-Crick model of DNA, the two strands had to be complementary. T had to be paired with A, and G with C. Therefore, if the sequence of bases on one strand was (for example) TTTGCTAAAGGTGAACCA... , then the other strand necessarily had to have the sequence AAACGATTTCCACTTGGT... The Watson-Crick model of DNA made it seem certain that all the genetic information needed for producing a new individual is coded into the long, thin, double-stranded DNA molecule of the cell nucleus, written in a four-letter language whose letters are the bases, adenine, thymine, guanine and cytosine.

The solution of the DNA structure in 1953 initiated a new kind of biology - molecular biology. This new discipline made use of recently-discovered physical techniques - X-ray diffraction, electron microscopy, electrophoresis, chromatography, ultracentrifugation, radioactive tracer techniques, autoradiography, electron spin resonance, nuclear magnetic resonance and ultraviolet spectroscopy. In the 1960's and 1970's, molecular biology became the most exciting and rapidly-growing branch of science.

2.4 The structure of DNA

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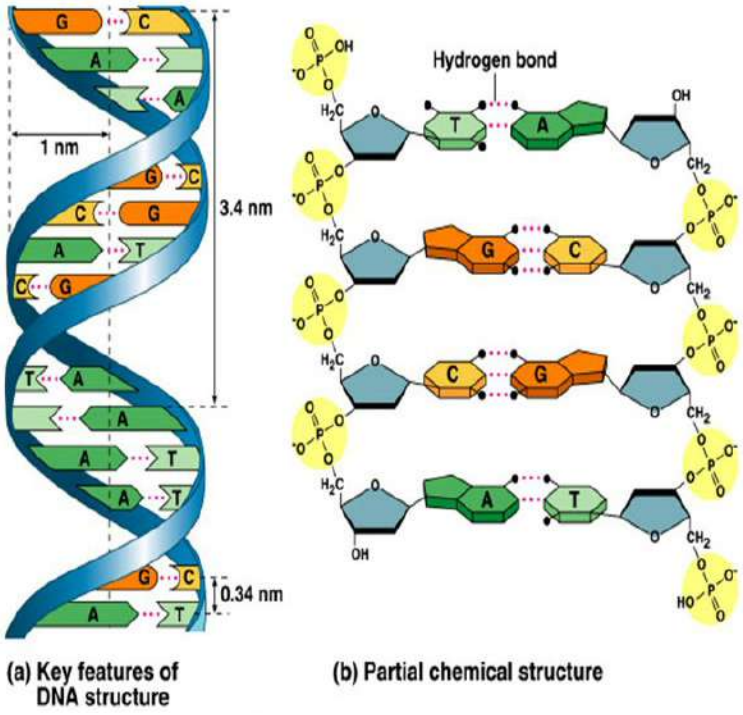

Figure 2.12: **Once the structure of DNA was known, it became clear that trans-generational information is transmitted in a chemical language based on a code with four letters, G, T, C and A.**

If the weak hydrogen bonds in the center of each rung were broken, the ladderlike DNA macromolecule could split down the center and divide into two single strands. Each single strand would then become a template for the formation of a new double-stranded molecule.

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2.5 RNA and ribosomes

Since DNA was known to carry the genetic message, coded into the sequence of the four nucleotide bases, A, T, G and C, and since proteins were known to be composed of specific sequences of the twenty amino acids, it was logical to suppose that the amino acid sequence in a protein was determined by the base sequence of DNA. The information somehow had to be read from the DNA and used in the biosynthesis of the protein.

It was known that, in addition to DNA, cells also contain a similar, but not quite identical, polynucleotide called ribonucleic acid (RNA). The sugar-phosphate backbone of RNA was known to differ slightly from that of DNA; and in RNA, the nucleotide thymine (T) was replaced by a chemically similar nucleotide, uracil (U). Furthermore, while DNA was found only in cell nuclei, RNA was found both in cell nuclei and in the cytoplasm of cells, where protein synthesis takes place. Evidence accumulated indicating that genetic information is first transcribed from DNA to RNA, and afterwards translated from RNA into the amino acid sequence of proteins.

At first, it was thought that RNA might act as a direct template, to which successive amino acids were attached. However, the appropriate chemical complementarity could not be found; and therefore, in 1955, Francis Crick proposed that amino acids are first bound to an adaptor molecule, which is afterward bound to RNA.

In 1956, George Emil Palade of the Rockefeller Institute used electron microscopy to study subcellular particles rich in RNA (ribosomes). Ribosomes were found to consist of two subunits - a smaller subunit, with a molecular weight one million times the weight of a hydrogen atom, and a larger subunit with twice this weight.

It was shown by means of radioactive tracers that a newly synthesized protein molecule is attached temporarily to a ribosome, but neither of the two subunits of the ribosome seemed to act as a template for protein synthesis. Instead, Palade and his coworkers found that genetic information is carried from DNA to the ribosome by a messenger RNA molecule (mRNA). Electron microscopy revealed that mRNA passes through the ribosome like a punched computer tape passing through a tape-reader. It was found that the adapter molecules, whose existence Crick had postulated, were smaller molecules of RNA; and these were given the name “transfer RNA” (tRNA). It was shown that, as an mRNA molecule passes through a ribosome, amino acids attached to complementary tRNA adaptor molecules are added to the growing protein chain.

The relationship between DNA, RNA, the proteins and the smaller molecules of a cell was thus seen to be hierarchical: The cell’s DNA controlled its proteins (through the agency of RNA); and the proteins controlled the synthesis and metabolism of the smaller molecules.

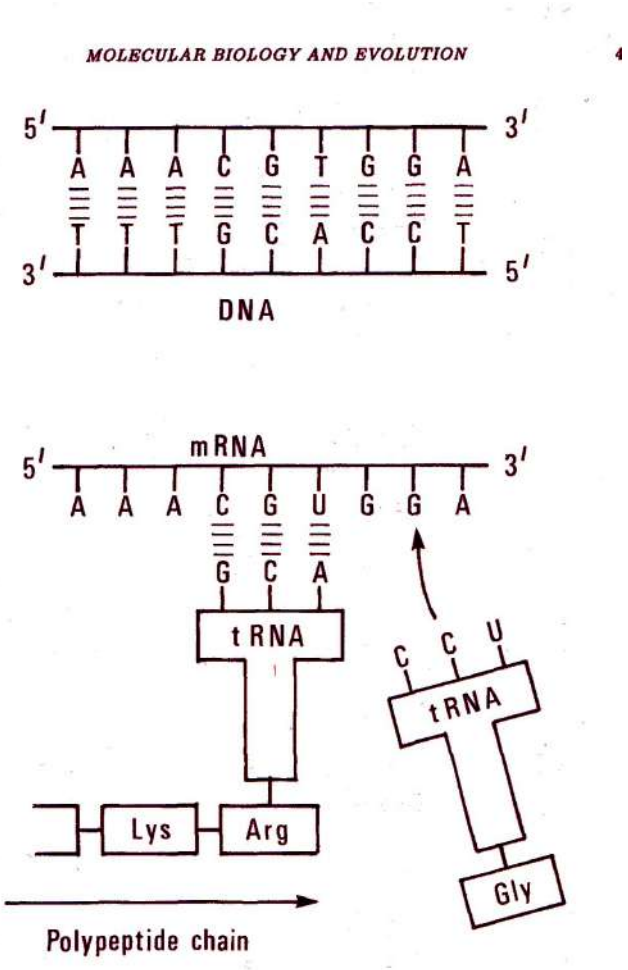

Figure 2.13: **Information coded on DNA molecules in the cell nucleus is transcribed to mRNA molecules. The messenger RNA molecules in turn provide information for the amino acid sequence in protein synthesis.**

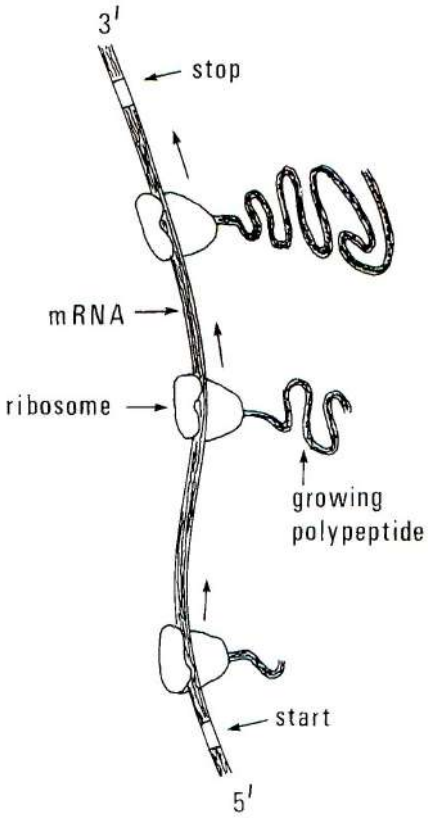

Figure 2.14: **mRNA passes through the ribosome like a punched computer tape passing through a tape-reader.**

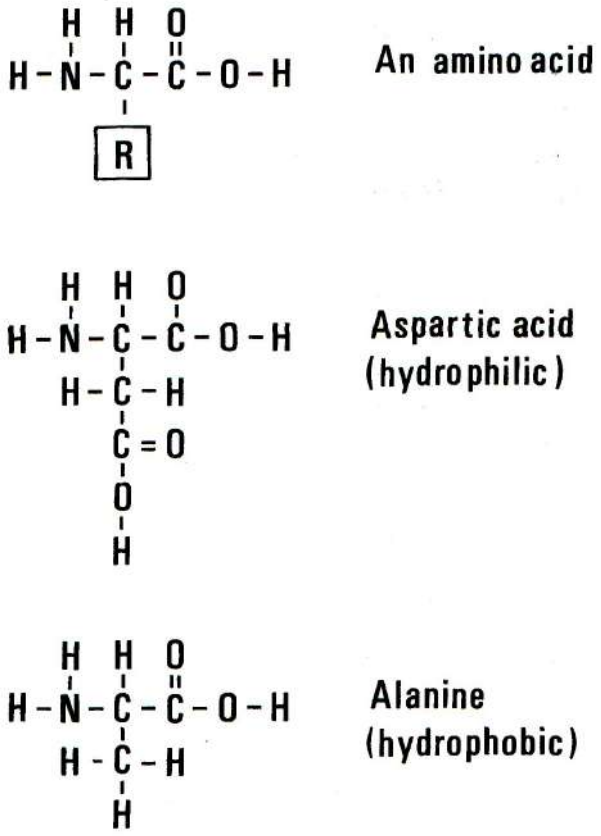

Figure 2.15: **This figure shows aspartic acid, whose residue (R) is hydrophilic, contrasted with alanine, whose residue is hydrophobic. A protein chain is formed from its constituent amino acids by removal of water so that a direct chain of the form -N-C-C-N-C-C-N-C-C-... is produced. The chain then folds in such a way that the hydrophilic residues are outermost while the hydrophobic residues are on the inside.**

2.6 The genetic code

In 1955, Severo Ochoa, at New York University, isolated a bacterial enzyme (RNA polymerase) which was able join the nucleotides A, G, U and C so that they became an RNA strand. One year later, this feat was repeated for DNA by Arthur Kornberg.

With the help of Ochoa's enzyme, it was possible to make synthetic RNA molecules containing only a single nucleotide - for example, one could join uracil molecules into the ribonucleic acid chain, ...U-U-U-U-U-U-... In 1961, Marshall Nirenberg and Heinrich Matthaei used synthetic poly-U as messenger RNA in protein synthesis; and they found that only polyphenylalanine was synthesized. In the same year, Sydney Brenner and Francis Crick reported a series of experiments on mutant strains of the bacteriophage, T4. The experiments of Brenner and Crick showed that whenever a mutation added or deleted either one or two base pairs, the proteins produced by the mutants were highly abnormal and non-functional. However, when the mutation added or subtracted three base pairs, the proteins often were functional. Brenner and Crick concluded that the genetic language has three-letter words (codons). With four different "letters", A, T, G and C, this gives sixty-four possible codons - more than enough to specify the twenty different amino acids.

In the light of the phage experiments of Brenner and Crick, Nirenberg and Matthaei concluded that the genetic code for phenylalanine is UUU in RNA and TTT in DNA. The remaining words in the genetic code were worked out by H. Gobind Khorana of the University of Wisconsin, who used other mRNA sequences (such as GUGUGU..., AAGAAGAAG... and GUUGUUGUU...) in protein synthesis. By 1966, the complete genetic code, specifying amino acids in terms of three-base sequences, was known. The code was found to be the same for all species studied, no matter how widely separated they were in form; and this showed that all life on earth belongs to the same family, as postulated by Darwin.

Table 2.1: **The genetic code**

TTT=Phe	TCT=Ser	TAT=Tyr	TGT=Cys
TTC=Phe	TCC=Ser	TAC=Tyr	TGC=Cys
TTA=Leu	TCA=Ser	TAA=Ter	TGA=Ter
TTG=Leu	TGC=Ser	TAG=Ter	TGG=Trp
CTT=Leu	CCT=Pro	CAT=His	CGT=Arg
CTC=Leu	CCC=Pro	CAC=His	CGC=Arg
CTA=Leu	CCA=Pro	CAA=Gln	CGA=Arg
CTG=Leu	CGC=Pro	CAG=Gln	CGG=Arg
ATT=Ile	ACT=Thr	AAT=Asn	AGT=Ser
ATC=Ile	ACC=Thr	AAC=Asn	AGC=Ser
ATA=Ile	ACA=Thr	AAA=Lys	AGA=Arg
ATG=Met	AGC=Thr	AAG=Lys	AGG=Arg
GTT=Val	GCT=Ala	GAT=Asp	GGT=Gly
GTC=Val	GCC=Ala	GAC=Asp	GGC=Gly
GTA=Val	GCA=Ala	GAA=Glu	GGA=Gly
GTG=Val	GGC=Ala	GAG=Glu	GGG=Gly

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Chapter 3

FLEMING, FLOREY AND CHAIN

3.1 Fleming

Education

Alexander Fleming was born in Ayrshire, Scotland in 1881, where his parents had a farm. Following in his elder brother's footsteps, he studied medicine, enrolling at St. Mary's Hospital Medical School in London. After serving in the Royal Army Medical Corps during World War I, he returned to St. Mary's, and was elected Professor of Bacteriology in 1928.

Treating the wounds of soldiers

While treating wounded soldiers during the First World War, Fleming had noticed that the antiseptics commonly applied to wounds did more harm than good. These antiseptics killed bacteria on the surface of wounds, but below, untouched by the antiseptics, anaerobic bacteria continued the infection, and the body's natural defenses were damaged by the antiseptics. Fleming published these observations, but the practice of treating wounds with strong antiseptics nevertheless continued.

The discovery of lysozyme

After the war, continuing his work at St. Mary's Hospital, Fleming searched for effective antibacterial substances. The first that he discovered was the enzyme lysozyme, which he found in the nasal secretions of a patient with a heavy cold. Working with lysozyme, he was disappointed to find that it was effective only against relatively harmless bacteria. In fact the reason those bacteria are harmless is that our bodies are already heavily armed with lysozyme. It occurs in tears, saliva, skin, hair and nails as well as mucus. In nature, egg whites contain large amounts of lysozyme.

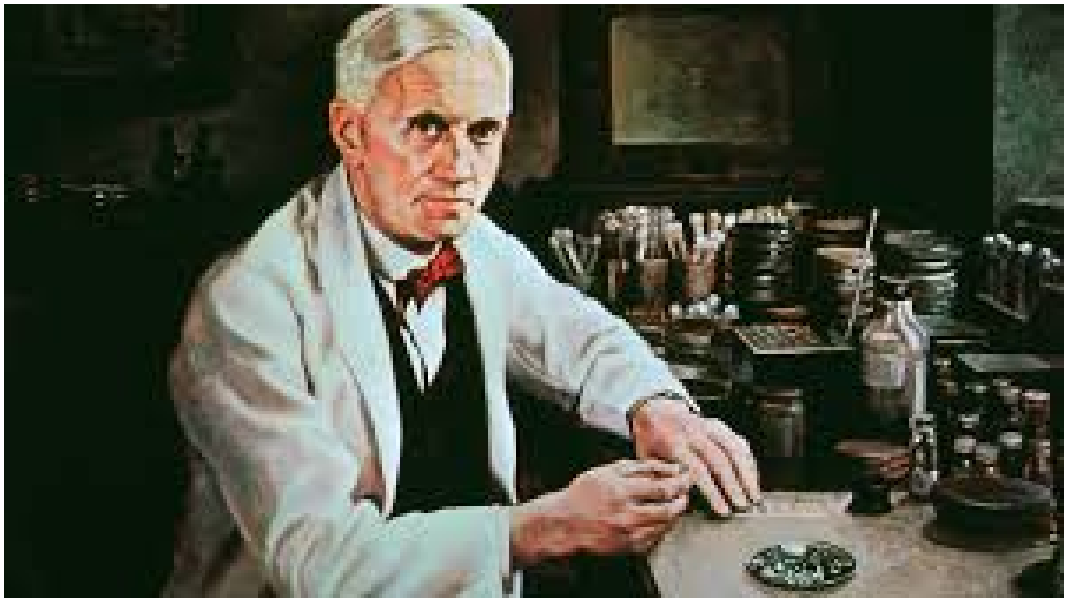

Figure 3.1: **Sir Alexander Fleming (1881-1955).**

The discovery of penicillin

"One sometimes finds, what one is not looking for. When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did." Alexander Fleming

Fleming was a brilliant researcher, but his laboratory was often messy. When he left with his family for a vacation in August, 1928, a jumble of petri dishes with staphylococci cultures were piled in a corner of the laboratory. Returning, a month later, Fleming noticed a mold growing in one of the culture dishes. Around the mold, the staphylococci were dead. He showed the dish to his former assistant, Merlyn Pryce. who said: "That's how you discovered lysozyme".

The Wikipedia article on the history of penicillin states that "The Scottish physician Alexander Fleming was the first to suggest that a Penicillium mold must secrete an antibacterial substance, and the first to concentrate the active substance involved, which he named penicillin, in 1928. Penicillin was the first modern antibiotic. During the next twelve years Fleming grew, distributed, and studied the original mold, which was determined to be a rare variant of Penicillium notatum (now Penicillium chrysogenum)."

Fleming was not the first person to suggest that molds could be used to treat infections. In fact the use of molds for this purpose has been known since ancient times. But it was Fleming's work that initiated the modern mass production and use of antibiotics.

Figure 3.2: **Fleming (center) receiving the Nobel prize from King Gustav V of Sweden (right) in 1945.**

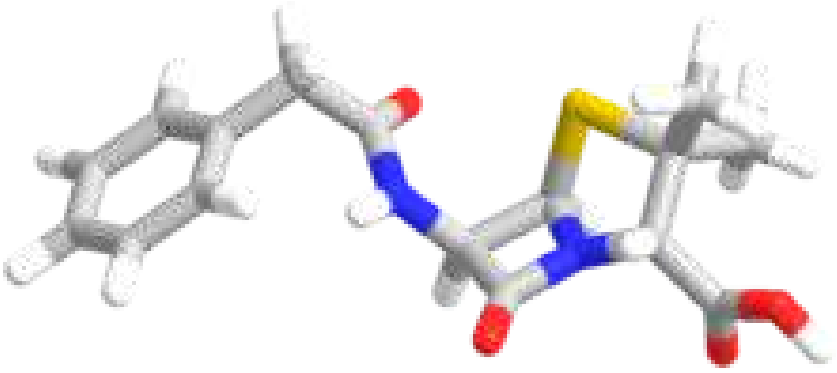

Figure 3.3: **3D-model of benzylpenicillin.**

Figure 3.4: **Faroe Islands postage stamp commemorating Fleming.**

Honors and awards

- Fleming, Florey and Chain jointly received the Nobel Prize in Medicine in 1945. According to the rules of the Nobel committee a maximum of three people may share the prize. Fleming's Nobel Prize medal was acquired by the National Museums of Scotland in 1989 and is on display after the museum re-opened in 2011.
- Fleming was a member of the Pontifical Academy of Sciences.
- Fleming was elected a Fellow of the Royal Society (FRS) in 1943.
- Fleming was awarded the Hunterian Professorship by the Royal College of Surgeons of England.
- Fleming was knighted, as a Knight Bachelor, by king George VI in 1944.
- He was made a Knight Grand Cross of the Order of Alfonso X the Wise in 1948.
- In 1999, Time magazine named Fleming one of the 100 Most Important People of the 20th century, statiimg: "It was a discovery that would change the course of history. The active ingredient in that mould, which Fleming named penicillin, turned out to be an infection-fighting agent of enormous potency. When it was finally recognized for what it was, the most efficacious life-saving drug in the world, penicillin would alter forever the treatment of bacterial infections. By the middle of the century, Fleming's discovery had spawned a huge pharmaceutical industry, churning out synthetic penicillin that would conquer some of mankind's most ancient scourges, including syphilis, gangrene and tuberculosis."

- The importance of his work was recognized by the placement of an International Historic Chemical Landmark plaque at the Alexander Fleming Laboratory Museum in London on November 19, 1999.
- When 2000 was approaching, at least three large Swedish magazines ranked penicillin as the most important discovery of the millennium.
- In 2002, Fleming was named in the BBC's list of the 100 Greatest Britons following a nationwide vote.
- A statue of Alexander Fleming stands outside the main bullring in Madrid, Plaza de Toros de Las Ventas. It was erected by subscription from grateful matadors, as penicillin greatly reduced the number of deaths in the bullring.
- FlemingovonámestÃ is a square named after Fleming in the university area of the Dejvice community in Prague.
- A secondary school is named after him in Sofia, Bulgaria.
- In Athens, a small square in the downtown district of Votanikos is named after Fleming and bears his bust. There are also a number of Streets in greater Athens and other towns in Greece named after either Fleming or his Greek second wife Amalia.
- In mid-2009, Fleming was commemorated on a new series of banknotes issued by the Clydesdale Bank; his image appears on the new issue of Â£5 notes.
- In 2009, Fleming was voted third greatest Scot in an opinion poll conducted by STV, behind only Scotland's national poet Robert Burns and national hero William Wallace.
- 91006 Fleming, an asteroid in the Asteroid Belt, is named after Fleming.
- Fleming station, on the Thessaloniki Metro system, takes its name from Fleming Street on which it is located, which in term is named after him.
- Sir Alexander Fleming College, a British school in Trujillo, northern Peru

3.2 Florey and Chain

Oxford University takes up the challenge

Alexander Fleming had been unable to produce large quantities of penicillin and to make it stable, so he became discouraged about the practical possibilities of using on a large scale as an antibacterial agent. However, a group of researchers at Oxford University in the department of the Professor of Pathology, Howard Florey, took up the challenge. Many researchers were involved in the effort to produce penicillin on a large scale and to make it in a stable form. At times the whole department was involved in the work, but the contributions of Ernst Boris Chain, Norman Heatley and Edward Abraham were especially important, especially those of Chain. In 1945 Chain shared the Nobel Prize in Physiology or Medicine with Fleming and Florey.

Figure 3.5: **Sir Howard Florey (1898-1968), later Lord Florey.**

Figure 3.6: **An Australian banknote with Florey's image.**

Figure 3.7: **Sir Ernst Boris Chain in 1945.**

Figure 3.8: **Ernst Chain in his laboratory.**

Figure 3.9: **Dr Ernst Chain undertakes an experiment in his office at the School of Pathology at Oxford University in 1944.**

3.3 War between micro-organisms

Antibiotics are the chemical weapons of microorganisms

Bacteria, viruses and molds do not live peacefully together. They are constantly at war. They kill each other with chemical weapons. Alexander Fleming was lucky enough to discover one of the chemical weapons with which molds fight against bacteria, but there are many many others. There are also extremely many chemical weapons used by bacteria to fight against each other. Finally some viruses, known as bacteriophages, attack and kill bacteria. Each of these cases offers humans new weapons in their fight against infectious disease.

The weapons of bacteria against other bacteria

If we grow cultures of two different species of bacteria on the same culture medium in a single petri dish, then often, after a few days, we will notice that one species of bacteria has died when it came in contact with the other. Such an event offers us the possibility of developing a new antibiotic. We merely need to culture, on a large scale, the bacterial strain that has successfully killed the other. Then we can isolate the active chemical agent. Research using the method just described is in progress to discover new antibiotics.

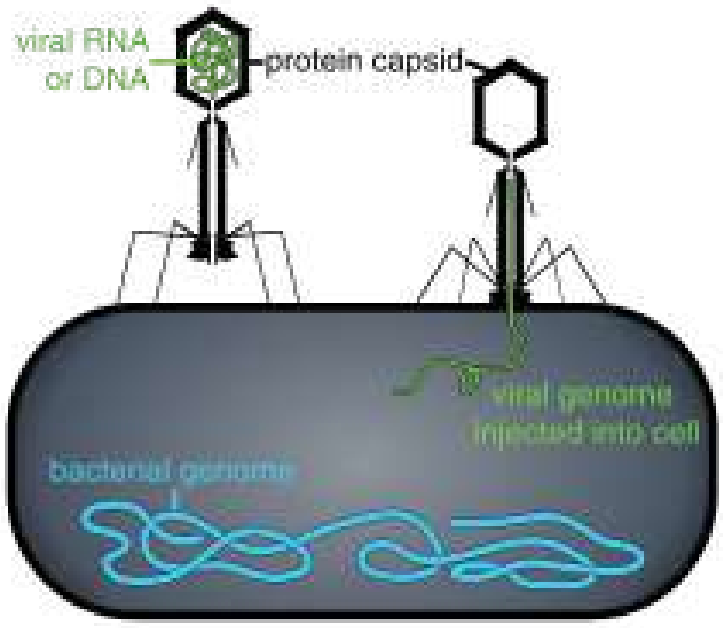

Figure 3.10: **Phage injecting its genome into bacterial cell.**

The use of bacteriophages in medicine

The Wikipedia article on Phage Therapy states that "The discovery of bacteriophages was reported by the Englishman Frederick Twort in 1915, and the French-Canadian Felix d'Hérelle in 1917. D'Hérelle said that the phages always appeared in the stools of *Shigella* dysentery patients shortly before they began to recover. He "quickly learned that bacteriophages are found wherever bacteria thrive: in sewers, in rivers that catch waste runoff from pipes, and in the stools of convalescent patients". Phage therapy was immediately recognized by many to be a key way forward for the eradication of pathogenic bacterial infections. A Georgian, George Eliava, was making similar discoveries. He travelled to the Pasteur Institute in Paris where he met d'Hérelle, and in 1923 he founded the Eliava Institute in Tbilisi, Georgia, devoted to the development of phage therapy. Phage therapy is used in Russia, Georgia and Poland...

"Isolated from Western advances in antibiotic production in the 1940s, Russian scientists continued to develop already successful phage therapy to treat the wounds of soldiers in field hospitals. During World War II, the Soviet Union used bacteriophages to treat many soldiers infected with various bacterial diseases e.g. dysentery and gangrene. Russian researchers continued to develop and to refine their treatments and to publish their research and results. However, due to the scientific barriers of the Cold War, this knowledge was not translated and did not proliferate across the world. A summary of these publications was published in English in 2009 in *A Literature Review of the Practical Application of Bacteriophage Research*."

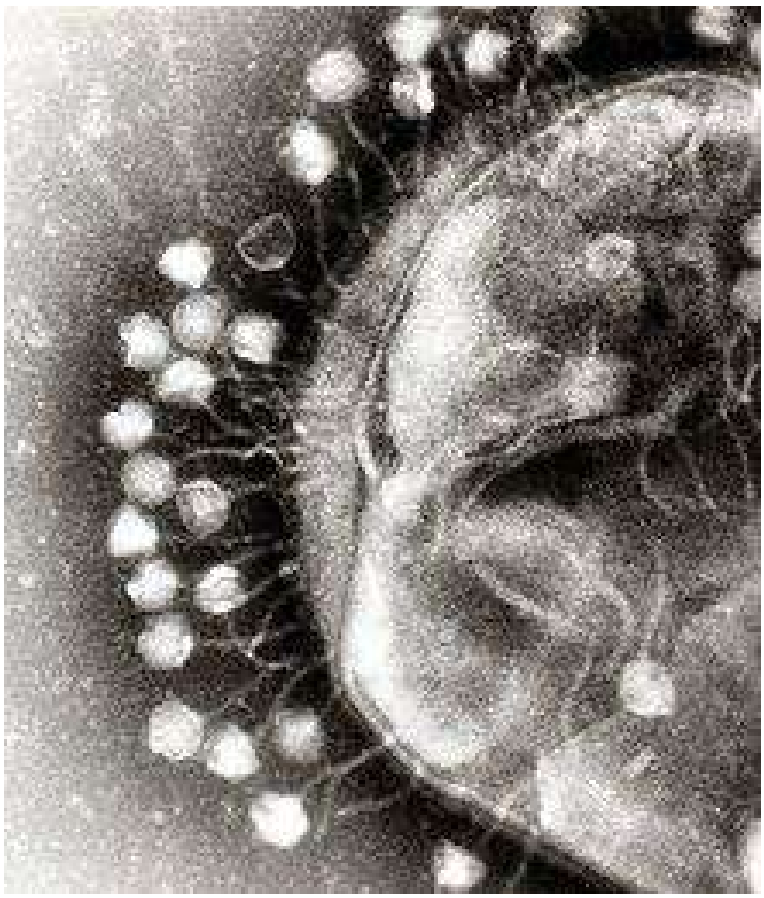

Figure 3.11: **An electron micrograph of bacteriophages attached to a bacterial cell. These viruses are the size and shape of coliphage T1.**

Figure 3.12: **Frederick Twort (1877-1950) discovered in 1915 that phages infect bacteria.**

Figure 3.13: **Félix d'Hérelle (1873-1949), co-discoverer of phages and pioneer of phage therapy.**

3.4 Overuse of antibiotics in agriculture

Pharming

A major global public health crisis may soon be produced by the wholesale use of antibiotics in the food of healthy farm animals. The resistance factors produced by shoveling antibiotics into animal food produces resistance factors (plasmids) which can easily be transferred to human pathogens. Pharming (instead of farming) is not a joke. It is a serious threat.[1]

Plasmids

Bacteria belong to a class of organisms (prokaryotes) whose cells do not have a nucleus. Instead, the DNA of the bacterial chromosome is arranged in a large loop

In the early 1950's, Joshua Lederberg discovered that bacteria can exchange genetic information. He found that a frequently-exchanged gene, the F-factor (which conferred fertility), was not linked to other bacterial genes; and he deduced that the DNA of the F-factor was not physically a part of the main bacterial chromosome. In 1952, Lederberg coined the word "plasmid" to denote any extrachromosomal genetic system.

In 1959, it was discovered in Japan that genes for resistance to antibiotics can be exchanged between bacteria; and the name "R-factors" was given to these genes. Like the F-factors, the R-factors did not seem to be part of the main loop of bacterial DNA. Because of the medical implications of this discovery, much attention was focused on the R-factors. It was found that they were plasmids, small loops of DNA existing inside the bacterial cell, but not attached to the bacterial chromosome. Further study showed that, in general, between one percent and three percent of bacterial genetic information is carried by plasmids, which can be exchanged freely even between different species of bacteria.

In the words of the microbiologist, Richard Novick, "Appreciation of the role of plasmids has produced a rather dramatic shift in biologists' thinking about genetics. The traditional view was that the genetic makeup of a species was about the same from one cell to another, and was constant over long periods of time. Now a significant proportion of genetic traits are known to be variable (present in some individual cells or strains, absent in others), labile (subject to frequent loss or gain) and mobile, all because those traits are associated with plasmids or other atypical genetic systems."

According to Ecowatch, "Roughly 80 percent of antibiotics purchased in the U.S. are fed to livestock to accelerate growth and prevent disease in healthy animals. Yet this

[1] http://ecowatch.com/2014/03/06/misuse-antibiotics-fatal-superbug-crisis/
http://ecowatch.com/2013/12/06/8-scary-facts-about-antibiotic-resistance/
http://ecowatch.com/2015/03/27/obama-fight-superbug-crisis/
http://ecowatch.com/2014/03/12/fda-regulation-antibiotics-factory-farms/
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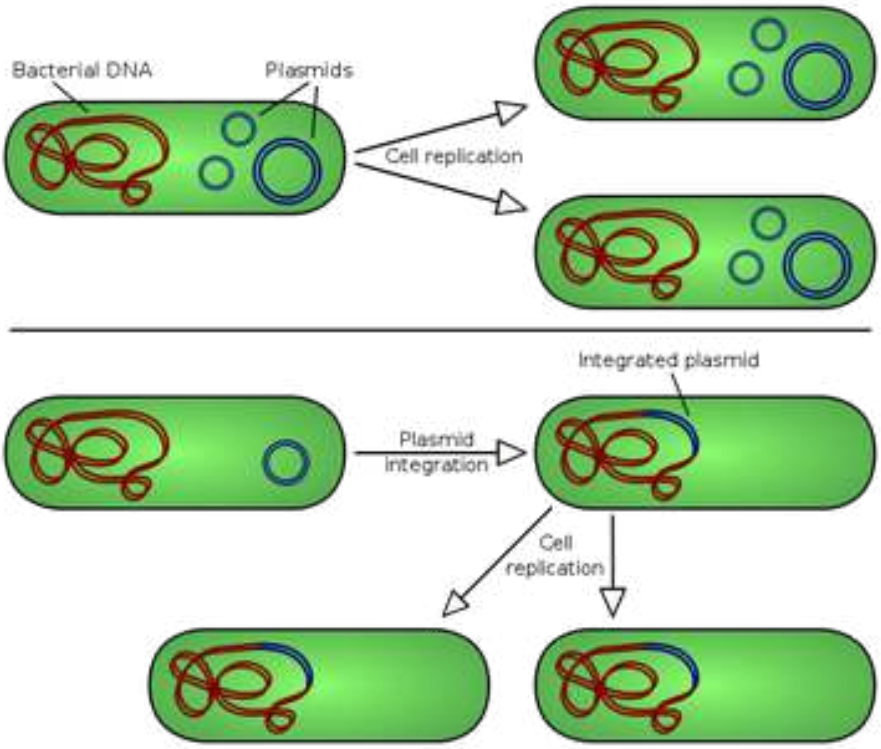

Figure 3.14: **There are two types of plasmid integration into a host bacteria: Non-integrating plasmids replicate as with the top instance, whereas episomes, the lower example, can integrate into the host.**

seemingly harmless practice also breeds superbugs, which can spread in the environment, contaminate food supplies and undermine the effectiveness of antibiotics.

"Antibiotic-resistant infections, like staphylococcus aureus, sicken at least 2 million Americans per year and kill more than 23,000, according to a 2013 CDC report. Those infections can happen anywhere, but they're especially deadly when they're spread in hospitals, nursing homes or other health care centers.

"Now the crisis is slowly worsening as drugmakers spend less time and money creating new antibiotics, even as more bacteria are becoming resistant to older drugs."

Suggestions for further reading

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Chapter 4

SZENT-GYÖRGYI

4.1 Summer work at Szent-Györgyi's laboratory

During the summers of 1960 and 1961, while I was still a postgraduate student in theoretical physics at the University of Chicago, I had the privilege of spending two summers working in the laboratory of the great Hungarian-American physiologist and biochemist, Albert Szent-Györgyi. He was famous for isolating vitamin C and for discovering the molecular mechanism of muscle contraction. But more importantly, he founded a new field of study: Bioenergetics.

Szent-Györgyi wondered how the chemical energy from food is harnessed to do mechanical work or to drive our metabolisms. He reasoned that there must be structures in living organisms which are analogous to the structures of engines. If you pour gasoline onto the street and set fire to it, no useful work results, only heat But if you burn it inside an engine, the chemical energy of the gasoline can be converted into useful mechanical work.

Following this line of thought, Szent-Györgyi looked for energy-transducing structures in the tissues of living organisms. Among the structures that caught Szent-Györgyi's attention were mitochondria, which power the metabolism of all animals, and he also studied the microscopic photosynthetic unit (thylakoids) in plants. After some years of work, he became convinced that quantum theory was needed in order to gain a complete understanding of how these microscopic engines work. Therefore he spent a year at the Institute for Advanced Study in Princeton, where he learned quite a lot of quantum theory.

Although he knew enough quantum theory to understand what physicists were talking about, he nevertheless thought that for the research which he wanted to undertake, he needed to collaborate with people whose whole education was in that field, and he brought some theoretical physicists (including me) to his laboratory. During the time that I was there, we worked to obtain a quantum theoretical understanding of the mechanism of the primary process in photosynthesis, where the energy of a photon is stabilized and trapped, ready to drive the synthesis of sugars.

I had heard about Albert Szent-Györgyi before the opportunity to work in his labora-

Figure 4.1: **Albert Szent-Györgyi in Italy in 1917.**

Figure 4.2: **Albert Szent-Györgyi in 1937, when he won the Nobel Prize in Physiology or Medicine. The prize was awarded partly for his work on the biochemistry of respiration, and partly for his isolation of vitamin C.**

Figure 4.3: **Szent-Györgyi working in his laboratory.**

tory presented itself. My brother Gordon had worked at the Woods Hole Marine Biological Laboratory during a previous summer and had told me that he considered Szent-Györgyi to be a great genius. Also, a University of Chicago classmate, David Freifelder, had said to me "You absolutely must read Szent-Györgyi's book, 'Bioenergetics'!"

4.2 Muscle contraction

Here are some excerpts from an article by Jack A. Roll, entitled *Generation of life in a test tube: Albert Szent-Györgyi, Bruno Straub, and the discovery of actin.* The article was published on 20 April, 2918 in Advances in Physiology Education[1]- Bruno Straub was Szent-Györgyi's student, with whom he collaborated on the work.

"Albert Szent-Györgyi, at 44 years of age, won the Nobel Prize in 1937 for his work on vitamin C and the establishment of the groundwork of the citric acid cycle. He now wanted to investigate one of the fundamental aspects of life and settled on the study of muscle contraction. The Szent-Györgyi laboratory in Hungary during World War II demonstrated that contraction could be reproduced in vitro by threads consisting of just two proteins, myosin and the newly discovered protein by Bruno Straub that they called actin. Szent-Györgyi called seeing the contraction of these threads, which occurred in the presence of ATP and ions, "the most thrilling moment" of his scientific life.

This major discovery of the generation of "life" in a test tube was totally unknown for years by the rest of the world because of the war. When the discovery was finally communicated to the world, it was not immediately accepted by all as being relevant to the physiology of muscle contraction.

4.3 Mitochondria

Mitochondria are believed to be descended from free-living bacteria. According to one theory for their evolution, they were engulfed and eaten by an ancient eukariotic cell, i.e. a large amoeba-like cell containing a nucleus and many organelles. The free-living bacteria thus eaten somehow escaped complete digestion and an endosymbiotic relationship was formed. This event may have occurred when the atmosphere of the earth changed from being reducing to oxidizing, because of the oxygen produced by plants. The benefit conferred by the symbiosis was the ability to perform oxidative phosphorolation, i.e. the synthesize ATP in an oxidizing atmosphere. Since that time, eukaryotes have contained mitochondria.

[1] https://www.physiology.org/doi/full/10.1152/advan.00189.2017

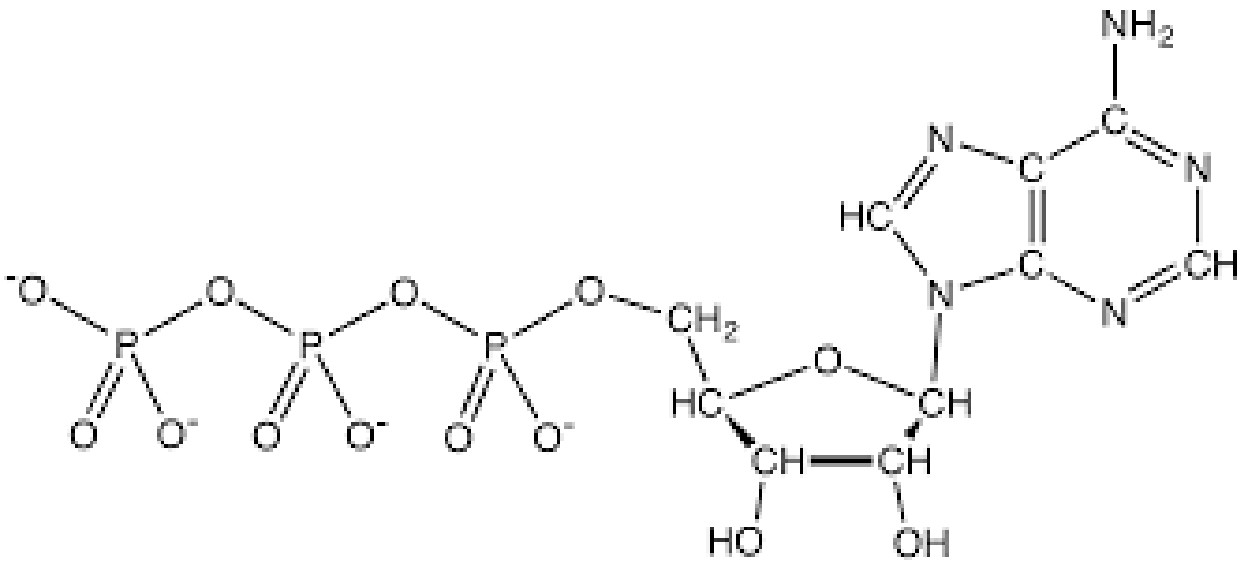

Figure 4.4: **The adinosine triphosphate (ATP) molecule acts as a universal fuel for both muscle contraction and metabolic processes within our bodies. Mitochondria use the stored chemical energy of sugars to synthesize ATP.**

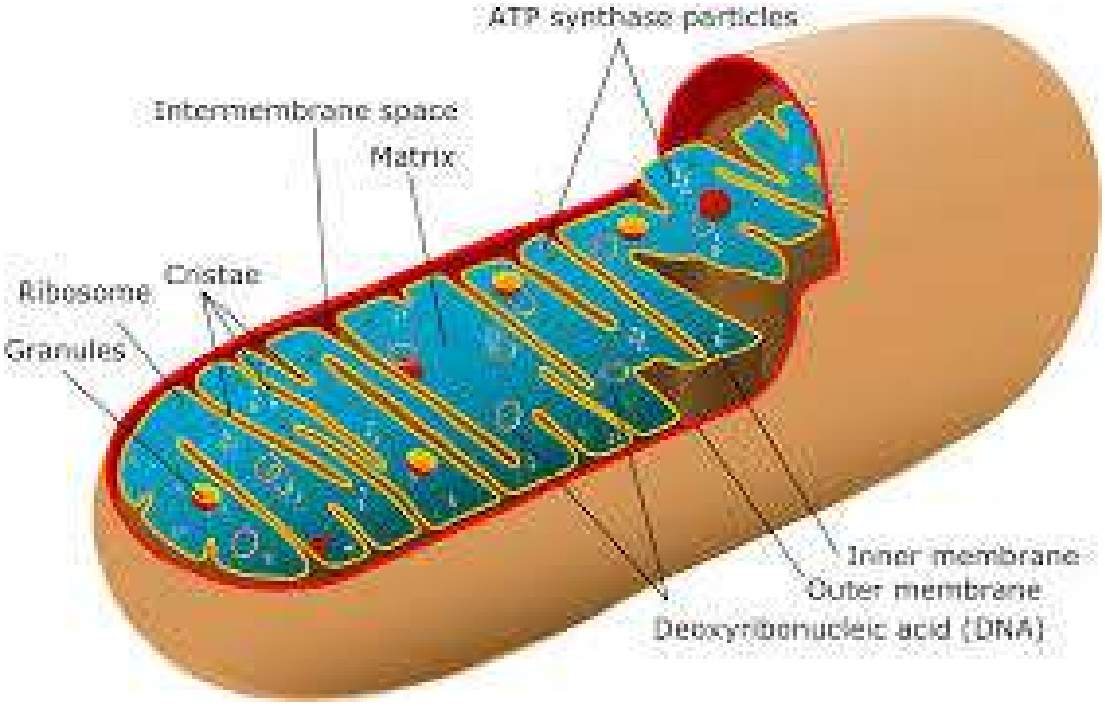

Figure 4.5: **Mitochondria contain membrane-bound enzymes that use the chemical energy of sugars to produce the high-energy phosphate bonds of adinosine triphosphate (ATP).**

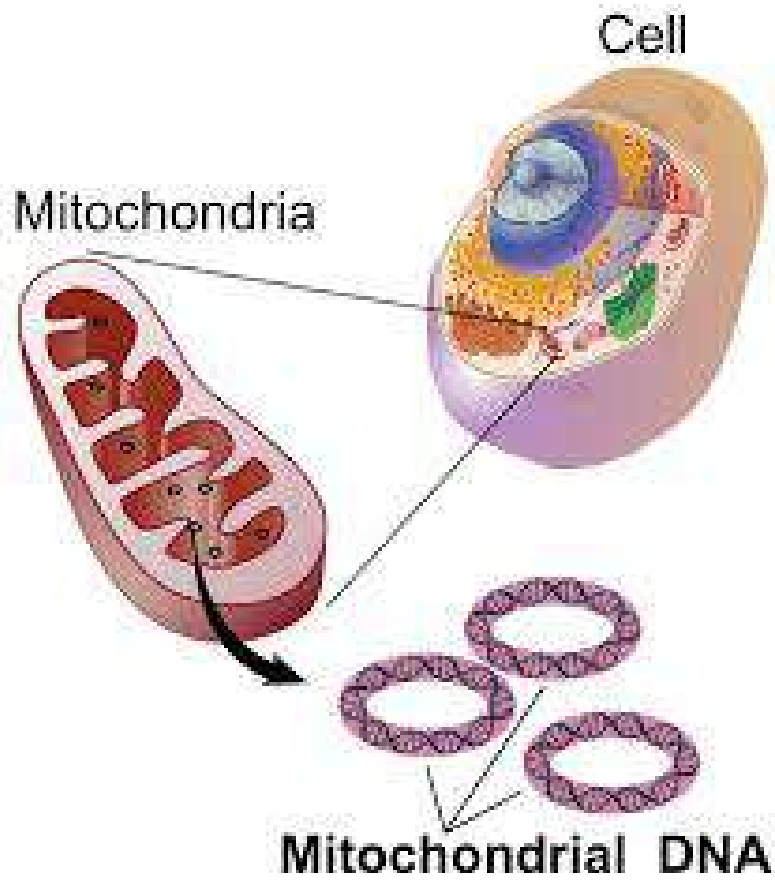

Figure 4.6: **Mitochondria are thought to be descended from free-living organisms, as is shown in Figure 12.6, and they have their own DNA.**

4.4 The photosynthetic unit

Like mitochondria, the chloroplasts that contain the photosynthetic unit of plants are thought to he the descendents of free-living cyanaobacteria, as is shown in Figure 12.6. Inside the chloroplasts are pocket-like structures called *thylakoids*. The membrane of thylakoids is like a sandwich. The middle part of this sandwich consists of pigment molecules, for example chloraphyl, which absorb the light, and produce an electron-hole pair. The outer layer of the thylakoid membrane sandwich consists of charge donor molecules, i.e. molecules whose highest filled molecular orbital is relatively high in energy, while the innermost layer consists of charge acceptor molecules, that is, molecules whose lowest empty orbital is quite low in energy. After a ohoton has been absorbed, the electron migrates to the charge.acceptors, while the hole migrates to the electron-donor molecules on the outside. Thus the electron and hole are rapidly separated, and the back-reaction is prevented. The mechanism is similar to the separation of the charge and hole in a silicon solar cell.

The Calvin cycle (the dark reaction)

After the primary process of photon absorption and charge-hole separation has taken place in the thylakoid, the available energy is stabilized in a dark reaction studies by Melvin Calvin (1911-1997) and his co.workers at the University of California, Berkeley. In the dark reaction, which is known as the *Calvin cycle*, the energy originally derived from absorption of a photon is further stabilized by being converted into the chemical energy of sugars. Calvin also contributed importantly to theories of the origin of life, and he is the author of a book entitles *Chemical Evolution Towards the Origin of Life On Earth and Elsewhere*. He was awarded the Nobel Prize for Chemistry in 1961.

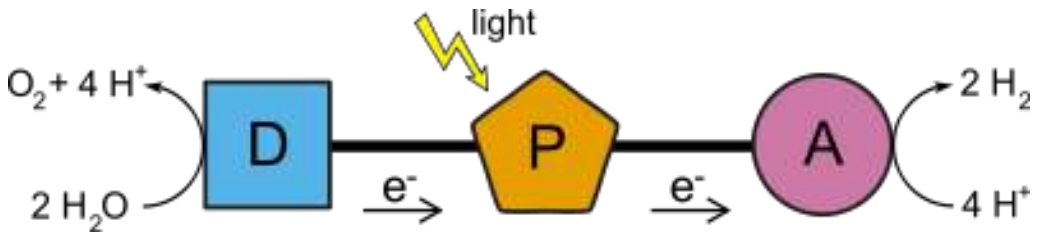

Figure 4.7: **The donor-pigment-acceptor triad needed for charge-hole separation.**

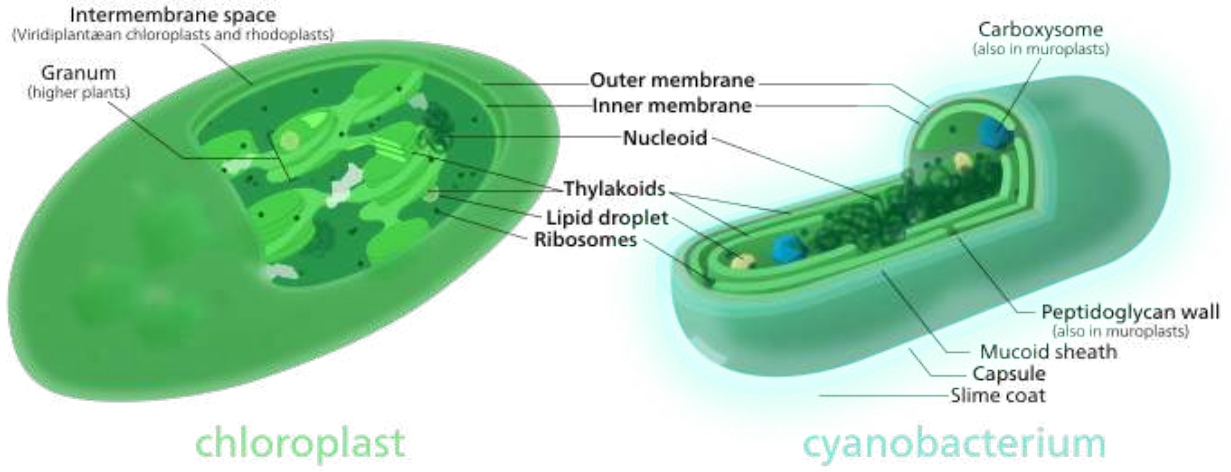

Figure 4.8: **Like mitochondria, chloroplasts were once free-living organisms, as is shown in Figure 12.6. Both chloroplasts and cyanobacteria have a double membrane, DNA, ribosomes, and thylakoids. Both the chloroplast and cyanobacterium depicted are idealized versions (the chloroplast is that of a higher plant) - a lot of diversity exists among chloroplasts and cyanobacteria.**

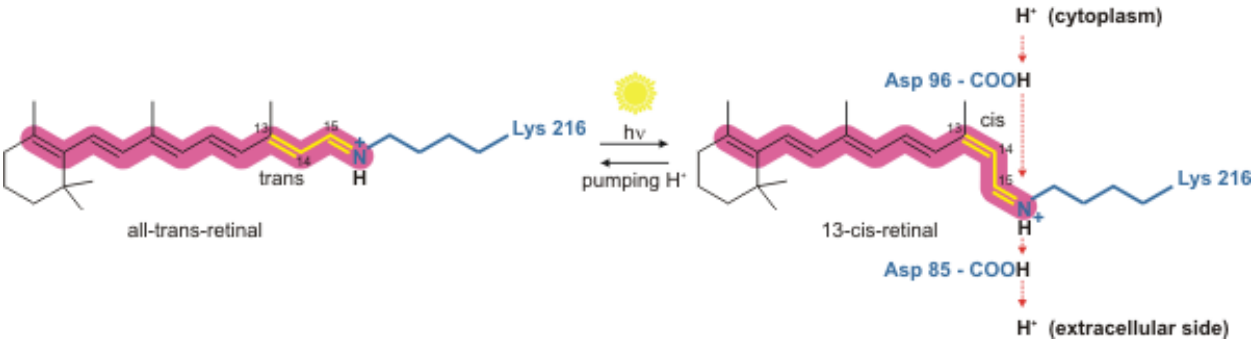

Figure 4.9: **Bacterial rhodopsin is interesting because it is a single molecule which is embedded in the membrane of the salt-loving bacterium *halobacterium halobium*, and which is capable of using the energy of sunlight to pump H^+ ions across the membrane against the electrochemical gradient. The molecule is almost identical to rhodopsin that occurs in our eyes. Perhaps, when our remote ancestors lived in the sea, they had a symbiotic relationship with halobacteria which led to the evolution of the vertebrate eye.**

4.5 Some of Albert Szent-Györgyi's personal reflections

On my Mother's side, I am the fourth generation of scientists. My Father was interested only in farming and so my Mother's influence prevailed. Music filled the house and the conversation at the table roamed about the intellectual achievements of the entire world. Politics and finance had no place in our thoughts. I am a scientist, myself, because at an early age I learned that only intellectual values were worth striving for, artistic or scientific creation being the highest aim. I strongly believe that we establish the coordinates of our evaluation at a very early age. What we do later depends on this scale of values which mostly cannot be changed later.

I wanted to understand life but found the complexity of physiology overÂ whelming. So I shifted to pharmacology where, at least, one of the partners, the drug, was simple. This, I found, did not relieve the difficulty. So, I went into bacteriology, but found bacteria too complex, too. I shifted on, to physicochemistry and then to chemistry, that is, to molecules, the smallest units in those days. Ten years ago I found molecules too complex and shifted to electrons, hoping to have reached bottom. But Nature has no bottom: its most basic principle is "organization." If Nature puts two things together she produces something new with new qualities, which cannot be expr essed in terms of qualities of the components. When going from electrons and protons to atoms, from here to molecules, molecular aggregates, etc., up to the cell or the whole animal, at every level we find something new, a new breathtaking vista. Whenever we separate two things, we lose something, something which may have been the most essential feature. So now, at 68, I am to work my way up again following electrons in their motion through more extensive systems, hoping to arrive, someday, at an understanding of the cellular level of organization. So the internal course of my life made a smooth sinusoid curve; not so the external course.

Lost in the 20th Century

Here are a few quotations from Albert Szent-Györgyi's autobiographical book, *Lost in the 20th Century*:

Overlooking my case history, I find a complete dichotomy. On the one hand, my inner story is exceedingly simple, if not indeed dull: my life has been devoted to science and my only real ambition has been to contribute to it and live up to its standards. In complete contradiction to this, the external course has been rather bumpy. I finished school in feudal Hungary as the son of a wealthy landowner and I had no worries about my future. A few

years later I find myself working in Hamburg, Germany, with a slight hunger edema. In 1942 I find myself in Istanbul, involved in secret diplomatic activÂ ity with a setting fit for a cheap and exciting spy story. Shortly after, I get a warning that Hitler had ordered the Governor of Hungary to appear before him, screaming my name at the top of his voice and demanding my delivery. Arrest warrants were passed out even against members of my family. In my pocket I find a Swedish passport, having been made a full Swedish citizen on the order of the King of Sweden-I am "Mr. Swenson," my wife, "M rs. Swenson." Sometime later I find myself in Moscow, treated in the most royal fashion by the Government (with caviar three times a day), but it does not take long before I am declared "a traitor of the people" and I play the role of the villain on the stages of Budapest. At the same time, I am refused entrance to the USA for my Soviet sympathies. Eventually, I find peace at Woods Hole, Massachusetts, working in a solitary corner of the Marine Biological Laboratory. After some nerve-racking complications, due to McCarthy, things straightened out, but the internal struggle is not comÂ pletely over . .I am troubled by grave doubts about the usefulness of scientific endeavor and have a whole drawer filled with treatises on politics and their relation to science, written for myself with the sole purpose of clarifying my mind, and finding an answer to the question: will science lead to the elevation or destruction of man, and has my scientific endeavor any sense? All this, in itself, would have no interest. There are many who did more for science, were braver, suffered more agony and even paid the penalty of death. What may lend interest to my story is that it reflects the turbulence of our days.

A fearless advocate of peace and rationality

Albert Szent-Györgyi spoke and wrote fearlessly against the institution of war. Here is a quotation from his writing:

The story of man consists of two parts, divided by the appearance of modern science... In the first period, man lived in the world in which his species was born and to which his senses were adapted. In the second, man stepped into a new, cosmic world to which he was a complete stranger... The forces at man's disposal were no longer terrestrial forces, of human dimension, but were cosmic forces, the forces which shaped the universe. The few hundred Fahrenheit degrees of our flimsy terrestrial fires were exchanged for the ten million degrees of the atomic reactions which heat the sun.

This is but a beginning, with endless possibilities in both directions; a building of a human life of undreamt of wealth and dignity, or a sudden end in utmost misery. Man lives in a new cosmic world for which he was not made. His survival depends on how well and how fast he can adapt himself to it, rebuilding all his ideas, all his social and political institutions.

...Modern science has abolished time and distance as factors separating nations. On our shrunken globe today, there is room for one group only: the family of man.

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Chapter 5

THE ORIGIN OF LIFE

5.1 Theories of chemical evolution towards the origin of life

The possibility of an era of chemical evolution prior to the origin of life entered the thoughts of Charles Darwin, but he considered the idea to be much too speculative to be included in his published papers and books. However, in February 1871, he wrote a letter to his close friend Sir Joseph Hooker containing the following words:

"It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sorts of ammonia and phosphoric salts, - light, heat, electricity etc. present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed."

The last letter which Darwin is known to have dictated and signed before his death in 1882 also shows that he was thinking about this problem: "You have expressed quite correctly my views", Darwin wrote, "where you said that I had intentionally left the question of the Origin of Life uncanvassed as being altogether ultra vires in the present state of our knowledge, and that I dealt only with the manner of succession. I have met with no evidence that seems in the least trustworthy, in favor of so-called Spontaneous Generation. (However) I believe that I have somewhere said (but cannot find the passage) that the principle of continuity renders it probable that the principle of life will hereafter be shown to be a part, or consequence, of some general law.."

Modern researchers, picking up the problem where Darwin left it, have begun to throw a little light on the problem of chemical evolution towards the origin of life. In the 1930's J.B.S. Haldane in England and A.I. Oparin in Russia put forward theories of an era of chemical evolution prior to the appearance of living organisms.

In 1924 Oparin published a pamphlet on the origin of life. An expanded version of this pamphlet was translated into English and appeared in 1936 as a book entitled *The Origin*

of Life on Earth. In this book Oparin pointed out that the time when life originated, conditions on earth were probably considerably different than they are at present: The atmosphere probably contained very little free oxygen, since free oxygen is produced by photosynthesis which did not yet exist. On the other hand, he argued, there were probably large amounts of methane and ammonia in the earth's primitive atmosphere[1]. Thus, before the origin of life, the earth probably had a reducing atmosphere rather than an oxidizing one. Oparin believed that energy-rich molecules could have been formed very slowly by the action of light from the sun. On the present-day earth, bacteria quickly consume energy-rich molecules, but before the origin of life, such molecules could have accumulated, since there were no living organisms to consume them. (This observation is similar to the remark made by Darwin in his 1871 letter to Hooker.)

The first experimental work in this field took place in 1950 in the laboratory of Melvin Calvin at the University of California, Berkeley. Calvin and his co-workers wished to determine experimentally whether the primitive atmosphere of the earth could have been converted into some of the molecules which are the building-blocks of living organisms. The energy needed to perform these conversions they imagined to be supplied by volcanism, radioactive decay, ultraviolet radiation, meteoric impacts, or by lightning strokes.

The earth is thought to be approximately 4.6 billion years old. At the time when Calvin and his co-workers were performing their experiments, the earth's primitive atmosphere was believed to have consisted primarily of hydrogen, water, ammonia, methane, and carbon monoxide, with a little carbon dioxide. A large quantity of hydrogen was believed to have been initially present in the primitive atmosphere, but it was thought to have been lost gradually over a period of time because the earth's gravitational attraction is too weak to effectively hold such a light and rapidly-moving molecule. However, Calvin and his group assumed sufficient hydrogen to be present to act as a reducing agent. In their 1950 experiments they subjected a mixture of hydrogen and carbon dioxide, with a catalytic amount of Fe^{2+}, to bombardment by fast particles from the Berkeley cyclotron. Their experiments resulted in a good yield of formic acid and a moderate yield of formaldehyde. (The fast particles from the cyclotron were designed to simulate an energy input from radioactive decay on the primitive earth.)

Two years later, Stanley Miller, working in the laboratory of Harold Urey at the University of Chicago, performed a much more refined experiment of the same type. In Miller's experiment, a mixture of the gases methane, ammonia, water and hydrogen was subjected to an energy input from an electric spark. Miller's apparatus was designed so that the gases were continuously circulated, passing first through the spark chamber, then through a water trap which removed the non-volatile water soluble products, and then back again through the spark chamber, and so on. The resulting products are shown as a function of time in Figure 3.5.

The Miller-Urey experiment produced many of the building-blocks of living organisms, including glycine, glycolic acid, sarcosine, alanine, lactic acid, N-methylalanine, β-alanine,

[1] It is now believed that the main constituents of the primordial atmosphere were carbon dioxide, water, nitrogen, and a little methane.

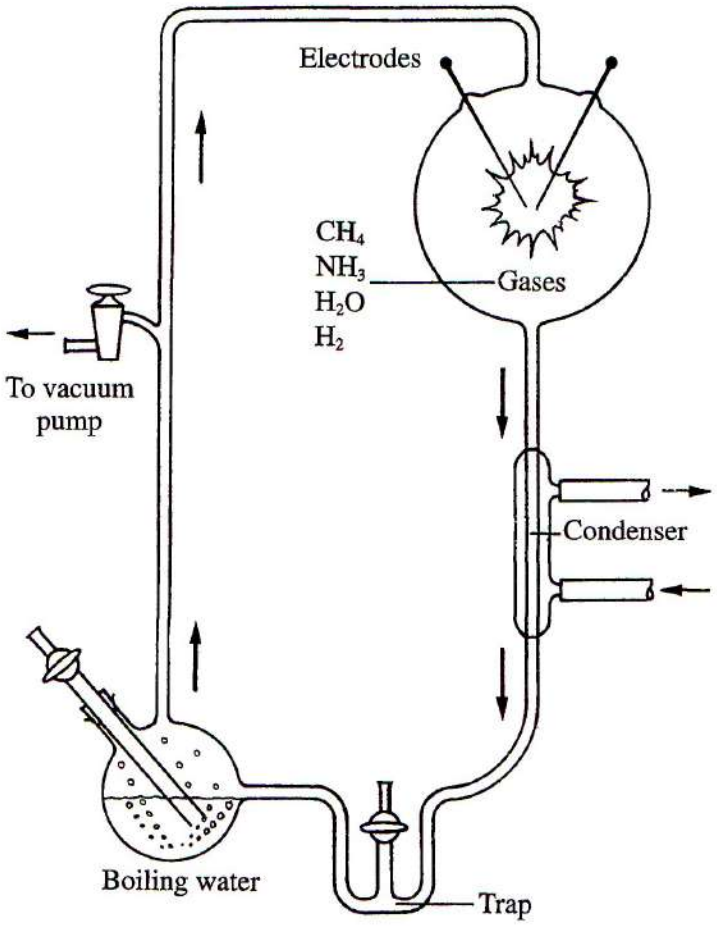

Figure 5.1: **Miller's apparatus.**

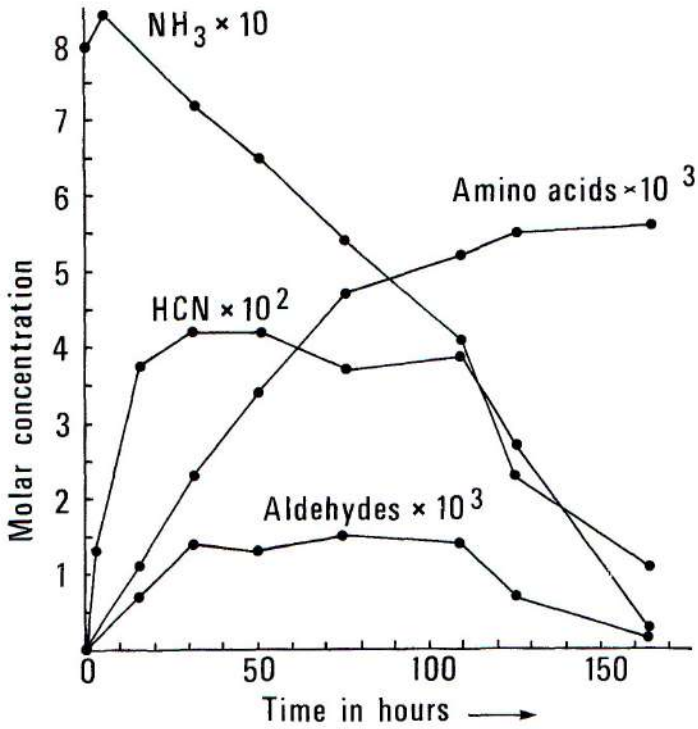

Figure 5.2: **Products as a function of time in the Miller-Urey experiment.**

succinic acid, aspartic acid, glutamic acid, iminodiacetic acid, iminoacetic-propionic acid, formic acid, acetic acid, propionic acid, urea and N-methyl urea[2]. Another major product was hydrogen cyanide, whose importance as an energy source in chemical evolution was later emphasized by Calvin.

The Miller-Urey experiment was repeated and extended by the Ceylonese-American biochemist Cyril Ponnamperuma and by the American expert in planetary atmospheres, Carl Sagan. They showed that when phosphorus is made available, then in addition to amino acids, the Miller-Urey experiment produces not only nucleic acids of the type that join together to form DNA, but also the energy-rich molecule ATP (adenosine triphosphate). ATP is extremely important in biochemistry, since it is a universal fuel which drives chemical reactions inside present-day living organisms.

Further variations on the Miller-Urey experiment were performed by Sydney Fox and his co-workers at the University of Miami. Fox and his group showed that amino acids can be synthesized from a primitive atmosphere by means of a thermal energy input, and that in the presence of phosphate esters, the amino acids can be thermally joined together to form polypeptides. However, some of the peptides produced in this way were cross linked, and hence not of biological interest.

In 1969, Melvin Calvin published an important book entitled *Chemical Evolution; Molecular Evolution Towards the Origin of Living Systems on Earth and Elsewhere*. In this book, Calvin reviewed the work of geochemists showing the presence in extremely ancient rock formations of molecules which we usually think of as being produced only by living organisms. He then discussed experiments of the Miller-Urey type - experiments simulating the first step in chemical evolution. According to Calvin, not only amino acids but also the bases adenine, thymine, guanine, cytosine and uracil, as well as various sugars, were probably present in the primitive ocean in moderate concentrations, produced from the primitive atmosphere by the available energy inputs, and not broken down because no organisms were present.

The next steps visualized by Calvin were dehydration reactions in which the building blocks were linked together into peptides, polynucleotides, lipids and porphyrins. Such dehydration reactions are in a thermodynamically uphill direction. In modern organisms, they are driven by a universally-used energy source, the high-energy phosphate bond of adenosine triphosphate (ATP). Searching for a substance present in the primitive ocean which could have driven the dehydrations, Calvin and his coworkers experimented with hydrogen cyanide (HC=N), and from the results of these experiments they concluded that the energy stored in the carbon-nitrogen triple bond of HC=N could indeed have driven the dehydration reactions necessary for polymerization of the fundamental building blocks. However, later work made it seem improbable that peptides could be produced from cyanide mixtures.

In Chemical Evolution, Calvin introduced the concept of autocatalysis as a mechanism for molecular selection, closely analogous to natural selection in biological evolution.

[2] The chemical reaction that led to the formation of the amino acids that Miller observed was undoubtedly the Strecker synthesis: $HCN + NH_3 + RC{=}O + H_2O \rightarrow RC(NH_2)COOH$.

Calvin proposed that there were a few molecules in the ancient oceans which could catalyze the breakdown of the energy-rich molecules present into simpler products. According to Calvin's hypothesis, in a very few of these reactions, the reaction itself produced more of the catalyst. In other words, in certain cases the catalyst not only broke down the energy-rich molecules into simpler products but also catalyzed their own synthesis. These autocatalysts, according to Calvin, were the first systems which might possibly be regarded as living organisms. They not only "ate" the energy-rich molecules but they also reproduced - i.e., they catalyzed the synthesis of molecules identical with themselves.

Autocatalysis leads to a sort of molecular natural selection, in which the precursor molecules and the energy-rich molecules play the role of "food", and the autocatalytic systems compete with each other for the food supply. In Calvin's picture of molecular evolution, the most efficient autocatalytic systems won this competition in a completely Darwinian way. These more efficient autocatalysts reproduced faster and competed more successfully for precursors and for energy-rich molecules. Any random change in the direction of greater efficiency was propagated by natural selection.

What were these early autocatalytic systems, the forerunners of life? Calvin proposed several independent lines of chemical evolution, which later, he argued, joined forces. He visualized the polynucleotides, the polypeptides, and the metallo-porphyrins as originally having independent lines of chemical evolution. Later, he argued, an accidental union of these independent autocatalysts showed itself to be a still more efficient autocatalytic system. He pointed out in his book that "autocatalysis" is perhaps too strong a word. One should perhaps speak instead of "reflexive catalysis" , where a molecule does not necessarily catalyze the synthesis of itself, but perhaps only the synthesis of a precursor. Like autocatalysis, reflexive catalysis is capable of exhibiting Darwinian selectivity.

The theoretical biologist, Stuart Kauffman, working at the Santa Fe Institute, has constructed computer models for the way in which the components of complex systems of reflexive catalysts may have been linked together. Kauffman's models exhibit a surprising tendency to produce orderly behavior even when the links are randomly programmed.

In 1967 and 1968, C. Woese, F.H.C. Crick and L.E. Orgel proposed that there may have been a period of chemical evolution involving RNA alone, prior to the era when DNA, RNA and proteins joined together to form complex self-reproducing systems. In the early 1980's, this picture of an "RNA world" was strengthened by the discovery (by Thomas R. Cech and Sydney Altman) of RNA molecules which have catalytic activity.

Today experiments aimed at throwing light on chemical evolution towards the origin of life are being performed in the laboratory of the Nobel Laureate geneticist Jack Sjostak at Harvard Medical School. The laboratory is trying to build a synthetic cellular system that undergoes Darwinian evolution.

In connection with autocatalytic systems, it is interesting to think of the polymerase chain reaction, which we discussed above. The target segment of DNA and the polymerase together form an autocatalytic system. The "food" molecules are the individual nucleotides in the solution. In the PCR system, a segment of DNA reproduces itself with an extremely high degree of fidelity. One can perhaps ask whether systems like the PCR system can have been among the forerunners of living organisms. The cyclic changes of temperature needed

for the process could have been supplied by the cycling of water through a hydrothermal system. There is indeed evidence that hot springs and undersea hydrothermal vents may have played an important role in chemical evolution towards the origin of life. We will discuss this evidence in the next section.

Throughout this discussion of theories of chemical evolution, and the experiments which have been done to support these theories, energy has played a central role. None of the transformations discussed above could have taken place without an energy source, or to be more precise, they could not have taken place without a source of free energy. In Chapter 4 we will discuss in detail the reason why free energy plays a central role, not only in the origin of life but also in life's continuation. We will see that there is a connection between free energy and information, and that information-containing free energy is needed to produce the high degree of order which is characteristic of life.

5.2 Molecular evidence establishing family trees in evolution

Starting in the 1970's, the powerful sequencing techniques developed by Sanger and others began to be used to establish evolutionary trees. The evolutionary closeness or distance of two organisms could be estimated from the degree of similarity of the amino acid sequences of their proteins, and also by comparing the base sequences of their DNA and RNA. One of the first studies of this kind was made by R.E. Dickerson and his coworkers, who studied the amino acid sequences in Cytochrome C, a protein of very ancient origin which is involved in the "electron transfer chain" of respiratory metabolism. Some of the results of Dickerson's studies are shown in Figure 12.6.

Comparison of the base sequences of RNA and DNA from various species proved to be even more powerful tool for establishing evolutionary relationships. Figure 12.7 shows the universal phylogenetic tree established in this way by Iwabe, Woese and their coworkers.[3] In Figure 12.7, all presently living organisms are divided into three main kingdoms, Eukaryotes, Eubacteria, and Archaebacteria. Carl Woese, who proposed this classification on the basis of comparative sequencing, wished to call the three kingdoms "Eucarya, Bacteria and Archaea". However, the most widely accepted terms are the ones shown in capital letters on the figure. Before the comparative RNA sequencing work, which was performed on the ribosomes of various species, it had not been realized that there are two types of bacteria, so markedly different from each other that they must be classified as belonging to separate kingdoms. One example of the difference between archaebacteria and eubacteria is that the former have cell membranes which contain ether lipids, while the latter have ester lipids in their cell membranes. Of the three kingdoms, the eubacteria and the archaebacteria are "prokaryotes", that is to say, they are unicellular organisms having no

[3] "Phylogeny" means "the evolutionary development of a species". "Ontogeny" means "the growth and development an individual, through various stages, for example, from fertilized egg to embryo, and so on." Ernst Haeckel, a 19th century follower of Darwin, observed that, in many cases, "ontogeny recapitulates phylogeny."

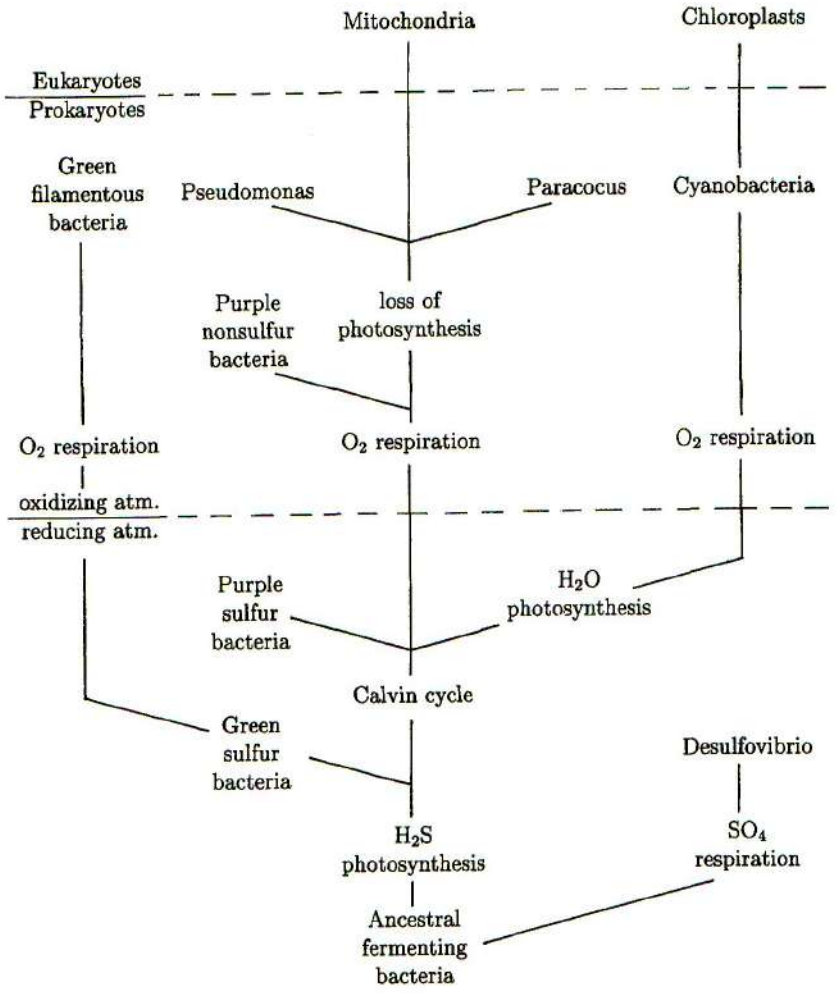

Figure 5.3: **Evolutionary relationships established by Dickerson and coworkers by comparing the amino acid sequences of Cytochrome C from various species.**

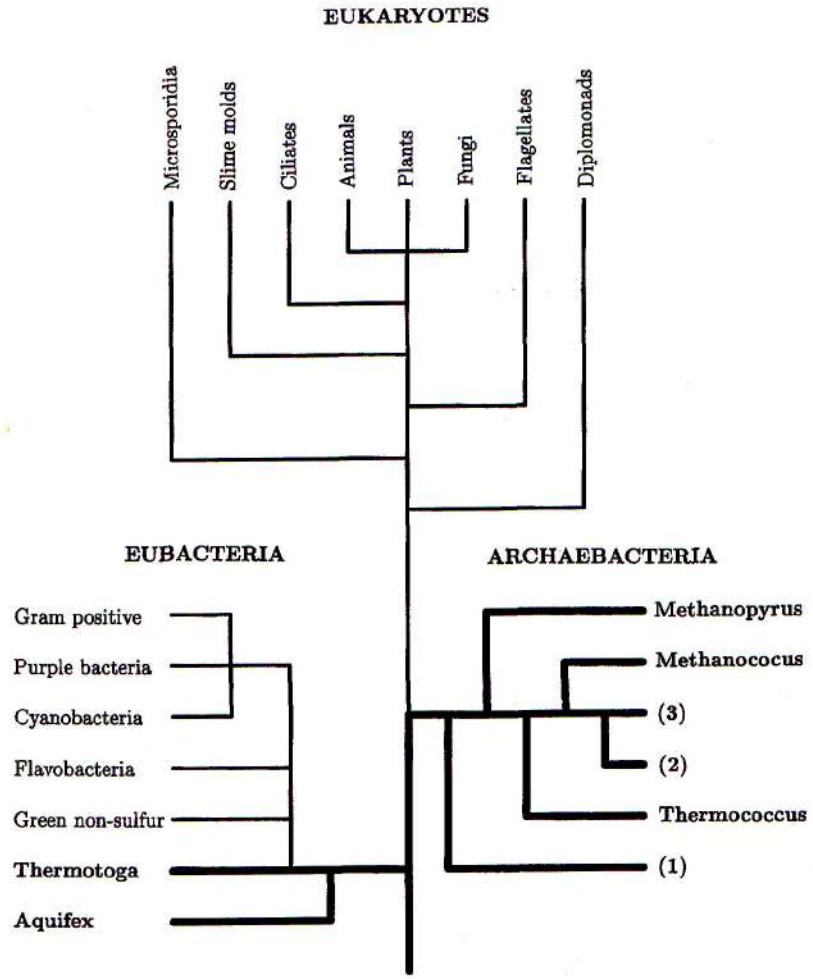

Figure 5.4: **This figure shows the universal phylogenetic tree, established by the work of Woese, Iwabe et al. Hyperthermophiles are indicated by bold lines and by bold type.**

cell nucleus. Most of the eukaryotes, whose cells contain a nucleus, are also unicellular, the exceptions being plants, fungi and animals.

One of the most interesting features of the phylogenetic tree shown in Figure 12.7 is that the deepest branches - the organisms with shortest pedigrees - are all hyperthermophiles, i.e. they live in extremely hot environments such as hot springs or undersea hydrothermal vents. The shortest branches represent the most extreme hyperthermophiles. The group of archaebacteria indicated by (1) in the figure includes **Thermofilum, Thermoproteus, Pyrobaculum, Pyrodictium, Desulfurococcus**, and **Sulfolobus** - all hypothermophiles[4]. Among the eubacteria, the two shortest branches, Aquifex and Thermatoga are both hyperthermophiles[5]

The phylogenetic evidence for the existence of hyperthermophiles at a very early stage of evolution lends support to a proposal put forward in 1988 by the German biochemist Günter Wächterhäuser. He proposed that the reaction for pyrite formation,

$$FeS + H_2S \rightarrow FeS_2 + 2H + +2e^-$$

which takes place spontaneously at high temperatures, supplied the energy needed to drive the first stages of chemical evolution towards the origin of life. Wächterhäuser pointed out that the surface of the mineral pyrite (FeS_2) is positively charged, and he proposed that, since the immediate products of carbon-dioxide fixation are negatively charged, they would be attracted to the pyrite surface. Thus, in Wächterhäuser's model, pyrite formation not only supplied the reducing agent needed for carbon-dioxide fixation, but also the pyrite surface aided the process. Wächterhäuser further proposed an archaic autocatylitic carbon-dioxide fixation cycle, which he visualized as resembling the reductive citric acid cycle found in present-day organisms, but with all reducing agents replaced by FeS + H_2S, with thioester activation replaced by thioacid activation, and carbonyl groups replaced by thioenol groups. The interested reader can find the details of Wächterhäuser's proposals in his papers, which are listed at the end of this chapter.

A similar picture of the origin of life has been proposed by Michael J. Russell and Alan J. Hall in 1997. In this picture "...(i) life emerged as hot, reduced, alkaline, sulphide-bearing submarine seepage waters interfaced with colder, more oxidized, more acid, $Fe^{2+} >> Fe^{3+}$-bearing water at deep (*ca.* 4km) floors of the Hadean ocean *ca.* 4 Gyr ago; (ii) the difference in acidity, temperature and redox potential provided a gradient of pH (*ca.* 4 units), temperature (*ca.* 60°C) and redox potential (*ca.* 500 mV) at the interface of those waters that was sustainable over geological time-scales, providing the continuity of conditions conducive to organic chemical reactions needed for the origin of life..." [6]. Russell, Hall and their coworkers also emphasize the role that may have been played by

[4] Group (2) in Figure 12.7 includes **Methanothermus**, which is hyperthermophilic, and Methanobacterium, which is not. Group (3) includes **Archaeoglobus**, which is hyperthermophilic, and Halococcus, Halobacterium, Methanoplanus, Methanospirilum, and Methanosarcina, which are not.

[5] Thermophiles are a subset of the larger group of extremophiles.

[6] See W. Martin and M.J. Russell, *On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells*, Philos. Trans. R. Soc. Lond. B Biol. Sci., **358**, 59-85, (2003).

Table 5.1: **Energy-yielding reactions of some lithoautotrophic hyperthermophiles. (After K.O. Setter)**

Energy-yielding reaction	Genera
$4H_2+CO_2 \rightarrow CH_4+2H_2O$	Methanopyrus, Methanothermus, Methanococcus
$H_2+S^\circ \rightarrow H_2S$	Pyrodictium, Thermoproteus, Pyrobaculum, Acidianus, Stygiolobus
$4H_2+H_2SO_4 \rightarrow H_2S+4H_2O$	Archaeoglobus

spontaneously-formed 3-dimensional mineral chambers (bubbles). They visualize these as having prevented the reacting molecules from diffusing away, thus maintaining high concentrations.

Table 12.1 shows the energy-yielding reactions which drive the metabolisms of some organisms which are of very ancient evolutionary origin. All the reactions shown in the table make use of H_2, which could have been supplied by pyrite formation at the time when the organisms evolved. All these organisms are lithoautotrophic, a word which requires some explanation: A heterotrophic organism is one which lives by ingesting energy-rich organic molecules which are present in its environment. By contrast, an autotrophic organism ingests only inorganic molecules. The lithoautotrophs use energy from these inorganic molecules, while the metabolisms of photoautotrophs are driven by energy from sunlight.

Evidence from layered rock formations called "stromatolites", produced by colonies of photosynthetic bacteria, show that photoautotrophs (or phototrophs) appeared on earth at least 3.5 billion years ago. The geological record also supplies approximate dates for other events in evolution. For example, the date at which molecular oxygen started to become abundant in the earth's atmosphere is believed to have been 2.0 billion years ago, with equilibrium finally being established 1.5 billion years in the past. Multi-cellular organisms appeared very late on the evolutionary and geological time-scale - only 600 million years ago. By collecting such evidence, the Belgian cytologist Christian de Duve has constructed the phylogenetic tree shown in Figure 12.8, showing branching as a function of time. One very interesting feature of this tree is the arrow indicating the transfer of "endosymbionts" from the eubacteria to the eukaryotes. In the next section, we will look in more detail at this important event, which took place about 1.8 billion years ago.

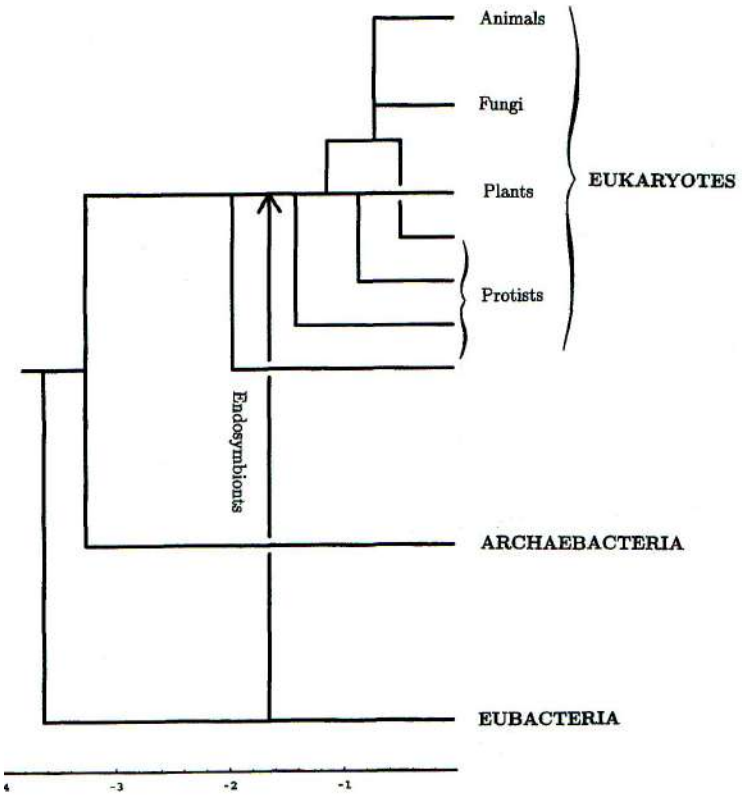

Figure 5.5: **Branching of the universal phylogenetic tree as a function of time. "Protists" are unicellular eukaryotes.**

5.3 Symbiosis

The word "symbiosis" is derived from Greek roots meaning "living together". It was coined in 1877 by the German botanist Albert Bernard Frank. By that date, it had become clear that lichens are composite organisms involving a fungus and an alga; but there was controversy concerning whether the relationship was a parasitic one. Was the alga held captive and exploited by the fungus? Or did the alga and the fungus help each other, the former performing photosynthesis, and the latter leeching minerals from the lichen's environment? In introducing the word "symbiosis" (in German, "Symbiotismus"), Prank remarked that "We must bring all the cases where two different species live on or in one another under a comprehensive concept which does not consider the role which the two individuals play but is based on the mere coexistence, and for which the term symbiosis is to be recommended." Thus the concept of symbiosis, as defined by Frank, included all intimate relationships between two or more species, including parasitism at one extreme and "mutualism" at the other. However, as the word is used today, it usually refers to relationships which are mutually beneficial.

Charles Darwin himself had been acutely aware of close and mutually beneficial relationships between organisms of different species. For example, in his work on the fertilization of flowers,he had demonstrated the way in which insects and plants can become exquisitely adapted to each other's needs. However, T.H. Huxley, "Darwin's bulldog", emphasized competition as the predominant force in evolution. "The animal world is on about the same level as a gladiator's show", Huxley wrote in 1888, "The creatures are fairly well treated and set to fight - whereby the strongest, the swiftest and the cunningest live to fight another day. The spectator has no need to turn his thumbs down, as no quarter is given." The view of nature as a sort of "gladiator's contest" dominated the mainstream of evolutionary thought far into the 20th century; but there was also a growing body of opinion which held that symbiosis could be an extremely important mechanism for the generation of new species.

Among the examples of symbiosis studied by Frank were the nitrogen-fixing bacteria living in nodules on the roots of legumes, and the mycorrhizal fungi which live on the roots of forest trees such as oaks, beech and conifers. Frank believed that the mycorrhizal fungi aid in the absorption of nutrients. He distinguished between "ectotrophic" fungi, which form sheaths around the root fibers, and "endotrophic" fungi, which penetrate the root cells. Other examples of symbiosis studied in the 19th century included borderline cases between plants and animals, for ex- ample, paramecia, sponges, hydra, planarian worms and sea anemones, all of which frequently contain green bodies capable of performing photosynthesis.

Writing in 1897, the American lichenologist Albert Schneider prophesied that "future studies may demonstrate that.., plasmic bodies (within the eukaryote cell), such as chlorophyll granules, leucoplastids, chromoplastids, chromosomes, centrosomes, nucleoli, etc., are perhaps symbionts comparable to those in less highly specialized symbiosis. Reinke expresses the opinion that it is not wholly unreasonable to suppose that some highly skilled scientist of the future may succeed in cultivating chlorophyll-bodies in artificial media."

19th century cytologists such as Robert Altman, Andreas Schimper and A. Benda focused attention on the chlorophyll-bodies of plants, which Schimper named chloroplasts, and on another type of subcellular granule, present in large numbers in all plant and animal cells, which Benda named mitochondria, deriving the name from the Greek roots mitos (thread) and chrondos (granule). They observed that these bodies seemed to reproduce themselves within the cell in very much the manner that might be expected if they were independent organisms. Schimper suggested that chloroplasts are symbionts, and that green plants owe their origin to a union of a colorless unicellular organism with a smaller chlorophyll-containing species.

The role of symbiosis in evolution continued to be debated in the 20th century. Mitochondria were shown to be centers of respiratory metabolism; and it was discovered that both mitochondria and chloroplasts contain their own DNA. However, opponents of their symbiotic origin pointed out that mitochondria alone cannot synthesize all their own proteins: Some mitochondrial proteins require information from nuclear DNA. The debate was finally settled in the 1970's, when comparative sequencing of ribosomal RNA in the laboratories of Carl Woese, W. Ford Doolittle and Michael Gray showed conclusively that both chloroplasts and mitochondria were originally endosymbionts. The ribosomal RNA sequences showed that chloroplasts had their evolutionary root in the cyanobacteria, a species of eubacteria, while mitochondria were traced to a group of eubacteria called the alpha-proteobacteria. Thus the evolutionary arrow leading from the eubacteria to the eukaryotes can today be drawn with confidence, as in Figure 3.8.

Cyanobacteria are bluish photosynthetic bacteria which often become linked to one another so as to form long chains. They can be found today growing in large colonies on seacoasts in many parts of the world, for example in Baja California on the Mexican coast. The top layer of such colonies consists of the phototrophic cyanobacteria, while the organisms in underlying layers are heterotrophs living off the decaying remains of the cyanobacteria. In the course of time, these layered colonies can become fosilized, and they are the source of the layered rock formations called stromatolites (discussed above). Geological dating of ancient stromatolites has shown that cyanobacteria must have originated at least 3.5 billion years ago.

Cyanobacteria contain two photosystems, each making use of a different type of chlorophyll. Photosystem I, which is thought to have evolved first, uses the energy of light to draw electrons from inorganic compounds, and sometimes also from organic compounds (but never from water). Photosystem II, which evolved later, draws electrons from water. Hydrogen derived from the water is used to produce organic compounds from carbon-dioxide, and molecular oxygen is released into the atmosphere. Photosystem II never appears alone. In all organisms which possess it, Photosystem II is coupled to Photosystem I, and together the two systems raise electrons to energy levels that are high enough to drive all the processes of metabolism. Dating of ancient stromatolites makes it probable that cyanobacteria began to release molecular oxygen into the earth's atmosphere at least 3.5 billion years ago; yet from other geological evidence we know that it was only 2 billion years ago that the concentration of molecular oxygen began to rise, equilibrium being reached 1.5 billion years ago. It is believed that ferrous iron, which at one time was

very abundant, initially absorbed the photosynthetically produced oxygen. This resulted in the time-lag, as well as the ferrous-ferric mixture of iron which is found in the mineral magnetite.

When the concentrations of molecular oxygen began to rise in earnest, most of the unicellular microorganisms living at the time found themselves in deep trouble, faced with extinction, because for them oxygen was a deadly poison; and very many species undoubtedly perished. However, some of the archaebacteria retreated to isolated anaerobic niches where we find them today, while others found ways of detoxifying the poisonous oxygen. Among the eubacteria, the ancestors of the alpha-proteobacteria were particularly good at dealing with oxygen and even turning it to advantage: They developed the biochemical machinery needed for respiratory metabolism.

Meanwhile, during the period between 3.5 and 2.0 billion years before the present, an extremely important evolutionary development had taken place: Branching from the archaebacteria, a line of large[7] heterotrophic unicellular organisms had evolved. They lacked rigid cell walls, and they could surround smaller organisms with their flexible outer membrane, drawing the victims into their interiors to be digested. These new heterotrophs were the ancestors of present-day eukaryotes, and thus they were the ancestors of all multicellular organisms.

Not only are the cells of present-day eukaryotes very much larger than the cells of archaebacteria and eubacteria; their complexity is also astonishing. Every eukaryote cell contains numerous intricate structures: a nucleus, cytoskeleton, Golgi apparatus, endoplasmic reticulum, mitochondria, peroxisomes, chromosomes, the complex structures needed for mitotic cell division, and so on. Furthermore, the genomes of eykaryotes contain very much more information than those of prokaryotes. How did this huge and relatively sudden increase in complexity and information content take place? According to a growing body of opinion, symbiosis played an important role in this development.

The ancestors of the eukaryotes were in the habit of drawing the smaller prokaryotes into their interiors to be digested. It seems likely that in a few cases the swallowed prokaryotes resisted digestion, multiplied within the host, were transmitted to future generations when the host divided, and conferred an evolutionary advantage, so that the result was a symbiotic relationship. In particular, both mitochondria and chloroplasts have definitely been proved to have originated as endosymbionts. It is easy to understand how the photosynthetic abilities of the chloroplasts (derived from cyanobacteria) could have conferred an advantage to their hosts, and how mitochondria (derived from alpha-proteobacteria) could have helped their hosts to survive the oxygen crisis. The symbiotic origin of other sub-cellular organelles is less well understood and is currently under intense investigation.

If we stretch the definition of symbiosis a little, we can make the concept include cooperative relationships between organisms of the same species. For example, cyanobacteria join together to form long chains, and they live together in large colonies which later turn into stromatolites. Also, some eubacteria have a mechanism for sensing how many of their species are present, so that they know, like a wolf pack, when it is prudent to attack a

[7] not large in an absolute sense, but large in relation to the prokaryotes

larger organism. This mechanism, called "quorum sensing", has recently attracted much attention among medical researchers.

The cooperative behavior of a genus of unicellular eukaryotes called slime molds is particularly interesting because it gives us a glimpse of how multicellular organisms may have originated. The name of the slime molds is misleading, since they are not fungi, but heterotrophic protists similar to amoebae. Under ordinary circumstances, the individual cells wander about independently searching for food, which they draw into their interiors and digest, a process called "phagocytosis". However, when food is scarce, they send out a chemical signal of distress. Researchers have analyzed the molecule which expresses slime mold unhappiness, and they have found it to be cyclic adenosine monophosphate (cAMP). At this signal, the cells congregate and the mass of cells begins to crawl, leaving a slimy trail. At it crawls, the community of cells gradually develops into a tall stalk, surmounted by a sphere - the "fruiting body". Inside the sphere, spores are produced by a sexual process. If a small animal, for example a mouse, passes by, the spores may adhere to its coat; and in this way they may be transported to another part of the forest where food is more plentiful.

Thus slime molds represent a sort of missing link between unicellular and multicellular or organisms. Normally the cells behave as individualists, wandering about independently, but when challenged by a shortage of food, the slime mold cells join together into an entity which closely resembles a multicellular organism. The cells even seem to exhibit altruism, since those forming the stalk have little chance of survival, and yet they are willing to perform their duty, holding up the sphere at the top so that the spores will survive and carry the genes of the community into the future. We should especially notice the fact that the cooperative behavior of the slime mold cells is coordinated by chemical signals.

Sponges are also close to the borderline which separates unicellular eukaryotes (protists) from multicellular organisms, but they are just on the other side of the border. Normally the sponge cells live together in a multicellular community, filtering food from water. However, if a living sponge is forced through a very fine cloth, it is possible to separate the cells from each other. The sponge cells can live independently for some time; but if many of them are left near to one another, they gradually join together and form themselves into a new sponge, guided by chemical signals. In a refinement of this experiment, one can take two living sponges of different species, separate the cells by passing the sponges through a fine cloth, and afterwards mix all the separated cells together. What happens next is amazing: The two types of sponge cells sort themselves out and become organized once more into two sponges - one of each species.

Slime molds and sponges hint at the genesis of multicellular organisms, whose evolution began approximately 600 million years ago. Looking at the slime molds and sponges, we can imagine how it happened. Some unicellular organisms must have experienced an enhanced probability of survival when they lived as colonies. Cooperative behavior and division of labor within the colonies were rewarded by the forces of natural selection, with the selective force acting on the entire colony of cells, rather than on the individual cell. This resulted in the formation of cellular societies and the evolution of mechanisms for cell differentiation. The division of labor within cellular societies (i.e., differentiation) came to

be coordinated by chemical signals which affected the transcription of genetic information and the synthesis of proteins. Each cell within a society of cells possessed the entire genome characteristic of the colony, but once a cell had been assigned its specific role in the economy of the society, part of the information became blocked - that is, it was not expressed in the function of that particular cell. As multicellular organisms evolved, the chemical language of intercellular communication became very much more complex and refined. We will discuss the language of intercellular communication in more detail in a later section.

Geneticists have become increasingly aware that symbiosis has probably played a major role in the evolution of multicellular organisms. We mentioned above that, by means of genetic engineering techniques, transgenic plants and animals can be produced. In these chimeras, genetic material from a foreign species is incorporated into the chromosomes, so that it is inherited in a stable, Mendelian fashion. J.A. Shapiro, one of whose articles is referenced at the end of this chapter, believes that this process also occurs in nature, so that the conventional picture of evolutionary family trees needs to be corrected. Shapiro believes that instead of evolutionary trees, we should perhaps think of webs or networks.

For example, it is tempting to guess that symbiosis may have played a role in the development of the visual system of vertebrates. One of the archaebacteria, the purple halobacterium halobium (recently renamed halobacterium salinarum), is able to perform photosynthesis by means of a protein called bacterial rhodopsin, which transports hydrogen ions across the bacterial membrane. This protein is a near chemical relative of rhodopsin, which combines with a carotinoid to form the "visual purple" used in the vertebrate eye. It is tempting to think that the close similarity of the two molecules is not just a coincidence, and that vertebrate vision originated in a symbiotic relationship between the photosynthetic halobacterium and an aquatic ancestor of the vertebrates, the host being able to sense when the halobacterium was exposed to light and therefore transporting hydrogen ions across its cell membrane.

In this chapter, we have looked at the flow of energy and information in the origin and evolution of life on earth. We have seen how energy-rich molecules were needed to drive the first steps in the origin of life, and how during the evolutionary process, information was preserved, transmitted, and shared between increasingly complex organisms, the whole process being driven by an input of energy. In the next chapter, we will look closely at the relationships between energy and information.

5.4 Timeline for the evolution of life on the Earth

The dates shown here are taken from the Wikipedia article entitled *Timeline of the evolutionary history of life*. The unit BYA means "Billion years ago", while MYA means "Million years ago".

- 4.540 BYA. Earliest Earth
- 4.404 BYA, First appearance of water on Earth.

- 4.280 BYA. Earliest appearance of life on Earth.[8]
- 3.900 BYA, Cells resembling prokaryotes appear. These first organisms use CO_2 as a source of carbon, and obtain energy by oxidizing inorganic materials.
- 3.500 BYA, Lifetime of the last universal common ancestor. The split between bacteria and archae occurs.
- 3.000 BYA, Photosynthetic cyanobacteria evolved. They used water as a reducing agent and produced oxygen as a waste product.
- 2.800 BYA, Earliest evidence of microbial life on land.
- 2.500 BYA, Great Oxygenation Event, produced by cyanobacteria's oxogenic photosynthesis.
- 1.850 BYA, Eukaryotic cells appear. They probably evolved from cooperative assemblages of prokaryotes (phagocytosis and symbiosis).
- 1.200 BYA, Sexual reproduction first appears in the fossil records. It may have existed earlier.
- 0.800 BYA, First multicellular organisms.
- 0.600 BYA, The ozone layer is formed, making landbased life more possible.
- 0.580-0.500 BYA, The Cambrian Explosion. Biodiversity quickly increases and most modern phyla of animals appear in the fossil record.
- 0.560 BYA, Fungi appear.
- 0.550 BYA, Comb jellies, sponges, sea anemones and corals evolved.
- 0.530 BYA, The first known fossilized footprints on land.
- 0.485 BYA, Jawless fishes.
- 0.434 BYA, The first primitive plants move onto land, accompanied by fungi which may have helped them.
- 0.420 BYA, Ray-finned fishes, arachnids, and land scorpions.
- 0.410 BYA, First signs of teeth in fish.
- 0.395 BYA, First lichens, stonewarts, harvestmen and springtails. The first known tracks of four-legged animals on land.
- 0.363 BYA, The Carboniferous Period starts. Insects appear on land and soon learn to fly. Seed-bearing plants and forests cover the land.
- 0.360 BYA, First crabs and ferns. Land flora dominated by ferns.
- 0.350 BYA, Large sharks, ratfishes and hagfish.
- 0.320 BYA, The precursors of mammals separate from the precursors to reptiles.
- 0.280 BYA, Earliest beetles, seed plants and conifers diversify.
- 0.2514 BYA, The Permian-Triassic extinction event eliminates 90-95% of marine species, and 70% of terrestrial vertebrates.[9]
- 0.245 BYA, Earliest icthyosaurs (i.e. seagoing dinosaurs).
- 0.225 BYA, Earliest dinosaurs. First mammals.

[8]This date for the first appearance of life on earth is earlier than previously thought possible. It is based on the ratio of carbon isotopes in zircon rocks recently found in Australia.

[9]Today, there is a danger that human use of fossil fuels will initiate a very similar extinction event. This danger will be discussed in a later chapter.

- 0.220 BYA, Seed-producing forests dominate the land. Herbivours grow to huge sizes. First flies and turtles.
- 0.155 BYA, First bloodsucking insects. Archaeopteryx, a possible ancestor of birds, appears.
- 0.130 BYA, Rise of the flowering plants. Coevolution of plants and their pollinators.
- 0.115 BYA, First monotreme (egg-laying) mammals.
- 0.110 BYA, Toothed diving birds.
- 0.100 BYA, Earliest bees.
- 0.090 BYA, Probable origin of placental mammals. However, the first undisputed fossil evidence is from 0.066 BYA.
- 0.080 BYA, First ants.
- 0.066 BYA, The Cretaceous-Paleogene extinction event wipes out about half of all animal species, including all of the dinosaurs except the birds. Afterwards, mammals become the dominant animal species. Conifers dominate northern forests.
- 0.060 BYA, Earliest true primates. Diversification of large, flightless birds. The ancestors of carnivorous mammals had appeared.
- 0.055 BYA, Diversification of birds. First songbirds, parrots, loons, swifts, and woodpeckers. First whale.
- 0.052 BYA, First bats appear in the fossil record.
- 0.050 BYA, Tapirs, rhinoceroses and camels appear. Diversification of primates.
- 0.040 BYA, Modern-type moths and butterflies were alive.
- 0.035 BYA, Grasses diversify. Many modern mammal groups appear.
- 0.030 BYA, Earliest pigs and cats.
- 0.025 BYA, First deer.
- 0.020 BYA, Giraffes, hyenas, bears, and giant anteaters appear. Birds increase in diversity.
- 0.015 BYA, First mastodons. Australian megafauna diversify. Kangaroos appear.
- 0.010 BYA, Grasslands and savannahs are established. Major diversification of grassland animals and snakes. Insects diversify, especially ants and termites.
- 0.0095 BYA = 9.50 MYA, Great American Interchange occurs. Armadillos, opossums, hummingbirds, "terror birds", and ground sloths were among the species that migrated from South America to North America after a land bridge formed between the previously isolated continents. Species moving in the opposite direction included horses, tapirs, saber-toothed cats, jaguars, bears, coaties, ferrets, otters, skunks and deer.
- 6.50 MYA, First homanins (our human ancestors diverging from the apes).
- 6.00 MYA, Australopithecines (extinct close relatives of humans after the split with chimpanzees) diversify.
- 5.00 MYA, First tree sloths and hippopotami. Diversification of grazing and carnivorous mammals.
- 4.00 MYA, Diversification of Australopithecines. The first modern elephants, giraffes, zebras, lions, rhinoceros and gazelles.

- 2.80 MYA, Appearance of a species intermediate between the Anthropithecines and Homo Habilis.
- 2.10 MYA, First member of the genus *Homo* appears, Homo habilis.

5.5 Life elsewhere in the universe

On December 18, 2017, scientists from the University of California published an article in *Science News* entitled *Ancient fossil microorganisms indicate that life in the universe is common*. According to the article:

"A new analysis of the oldest known fossil microorganisms provides strong evidence to support an increasingly widespread understanding that life in the universe is common.

"The microorganisms, from Western Australia, are 3.465 billion years old. Scientists from UCLA and the University of Wisconsin-Madison report today in the journal Proceedings of the National Academy of Sciences that two of the species they studied appear to have performed a primitive form of photosynthesis, another apparently produced methane gas, and two others appear to have consumed methane and used it to build their cell walls.

"The evidence that a diverse group of organisms had already evolved extremely early in the Earth's history, combined with scientists' knowledge of the vast number of stars in the universe and the growing understanding that planets orbit so many of them, strengthens the case for life existing elsewhere in the universe because it would be extremely unlikely that life formed quickly on Earth but did not arise anywhere else."

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Chapter 6

HODGKIN, HUXLEY AND ECCLES

6.1 The flow of information between and within cells

Information is transferred between cells in several ways. Among bacteria, in addition to the chronologically vertical transfer of genetic information directly from a single parent to its two daughter cells on cell division, there are mechanisms for the sharing of genetic information in a chronologically horizontal way, between cells of the same generation. These horizontal genetic information transfers can be thought of as being analogous to sex, as will be seen more clearly from some examples.

In the most primitive mechanism of horizontal information transfer, a bacterium releases DNA into its surroundings, and the DNA is later absorbed by another bacterium, not necessarily of the same species. For example, a loop or plasmid of DNA conferring resistance to an antibiotic (an "R-factor") can be released by a resistant bacterium and later absorbed by a bacterium of another species, which then becomes resistant[1].

A second mechanism for horizontal information transfer involves infection of a bacterium by a virus. As the virus reproduces itself inside the bacterium, some of the host's DNA can chance to be incorporated in the new virus particles, which then carry the extra DNA to other bacteria.

Finally, there is a third mechanism (discovered by J. Lederberg) in which two bacteria come together and construct a conjugal bridge across which genetic information can flow.

Almost all multicellular animals and plants reproduce sexually. In the case of sexual reproduction the genetic information of both parents is thrown into a lottery by means of special cells, the gametes. Gametes of each parent contain only half the genetic information

[1] The fact that this can happen is a strong reason for using antibiotics with great caution in agriculture. Resistance to antibiotics can be transferred from the bacteria commonly found in farm animals to bacteria which are dangerous for humans. Microbiologists have repeatedly warned farmers, drug companies and politicians of this danger, but the warnings have usually been ignored. Unfortunately there are now several instances of antibiotic-resistant human pathogens that have been produced by indiscriminate use of antibiotics in agriculture.

of the parent, and the exact composition of that half is determined by chance. Thus, when the gametes from two sexes fuse to form a new individual, the chances for variability are extremely large. This variability is highly valuable to multicellular organisms which reproduce sexually, not only because variability is the raw material of evolutionary adaption to changes in the environment, but also because the great variability of sexually-reproducing organisms makes them less likely to succumb to parasites. Infecting bacteria might otherwise deceive the immune systems of their hosts by developing cell-surface antigens which resemble those of the host, but when they infect sexually-reproducing organisms where each individual is unique, this is much less likely.

Within the cells of all organisms living today, there is a flow of information from polynucleotides (DNA and RNA) to proteins. As messenger RNA passes through a ribosome, like punched tape passing through a computer tapereader, the sequence of nucleotides in the mRNA is translated into the sequence of nucleic acids in the growing protein. The molecular mechanism of the reading and writing in this process involves not only spatial complementarity, but also complementarity of charge distributions.

As a protein grows, one amino acid at a time, it begins to fold. The way in which it folds (the "tertiary conformation") is determined both by spatial complementarity and by complementarity of charge distributions: Those amino acids which have highly polar groups, i.e., where several atoms have large positive or negative excess charges - "hydrophilic" amino acids - tend to be placed on the outside of the growing protein, while amino acids lacking large excess charges - "hydrophobic" amino acids - tend to be on the inside, away from water. Hydrophilic amino acids form hydrogen bonds with water molecules. Whenever there is a large negative charge on an atom of an amino acid, it attracts a positively-charged hydrogen from water, while positively-charged hydrogens on nucleic acids are attracted to negatively charged oxygens of water. Meanwhile, in the interior of the growing protein, non-polar amino acids are attracted to each other by so-called van der Waals forces, which do not require large excess charges, but only close proximity.

When a protein is complete, it is ready to participate in the activities of the cell, perhaps as a structural element or perhaps as an enzyme. Enzymes catalyze the processes by which carbohydrates, and other molecules used by the cell, are synthesized. Often an enzyme has an "active site", where such a process takes place. Not only the spatial conformation of the active site but also its pattern of excess charges must be right if the catalysis is to be effective. An enzyme sometimes acts by binding two smaller molecules to its active site in a proper orientation to allow a reaction between them to take place. In other cases, substrate molecules are stressed and distorted by electrostatic forces as they are pulled into the active site, and the activation energy for a reaction is lowered.

Thus, information is transferred first from DNA and RNA to proteins, and then from proteins to (for example) carbohydrates. Sometimes the carbohydrates then become part of surface of a cell. The information which these surface carbohydrates ("cell surface antigens") contain may be transmitted to other cells. In this entire information transfer process, the "reading" and "writing" depend on steric complementarity and on complementarity of molecular charge distributions.

Not only do cells communicate by touching each other and recognizing each other's cell

surface antigens - they also communicate by secreting and absorbing transmitter molecules. For example, the group behavior of slime mold cells is coordinated by the cyclic adenosine monophosphate molecules, which the cells secrete when distressed.

Within most multicellular organisms, cooperative behavior of cells is coordinated by molecules such as hormones - chemical messengers. These are recognized by "receptors", the mechanism of recognition once again depending on complementarity of charge distributions and shape. Receptors on the surfaces of cells are often membrane-bound proteins which reach from the exterior of the membrane to the interior. When an external transmitter molecule is bound to a receptor site on the outside part of the protein, it causes a conformational change which releases a bound molecule of a different type from a site on the inside part of the protein, thus carrying the signal to the cell's interior. In other cases the messenger molecule passes through the cell membrane.

In this way the individual cell in a society of cells (a multicellular organism) is told when to divide and when to stop dividing, and what its special role will be in the economy of the cell society (differentiation). For example, in humans, follicle-stimulating hormone, lutenizing hormone, prolactin, estrogen and progesterone are among the chemical messengers which cause the cell differentiation needed to create the secondary sexual characteristics of females.

Another role of chemical messengers in multicellular organisms is to maintain a reasonably constant internal environment in spite of drastic changes in the external environment of individual cells or of the organism as a whole (homeostasis). An example of such a homeostatic chemical messenger is the hormone insulin, which is found in humans and other mammals. The rate of its release by secretory cells in the pancreas is increased by high concentrations of glucose in the blood. Insulin carries the news of high glucose levels to target cells in the liver, where the glucose is converted to glycogen, and to other target cells in the muscles, where the glucose is burned.

6.2 Nervous systems

Hormones require a considerable amount of time to diffuse from the cells where they originate to their target cells; but animals often need to act very quickly, in fractions of seconds, to avoid danger or to obtain food. Because of the need for quick responses, a second system of communication has evolved - the system of neurons.

Neurons have a cell bodies, nuclei, mitochondria and other usual features of eukaryotic cells, but in addition they possess extremely long and thin tubelike extensions called axons and dendrites. The axons function as informational output channels, while the dendrites are inputs. These very long extensions of neurons connect them with other neurons which can be at distant sites, to which they are able to transmit electrical signals. The complex network of neurons within a multicellular organism, its nervous system, is divided into three parts. A sensory or input part brings in signals from the organism's interior or from its external environment. An effector or output part produces a response to the input signal, for example by initiating muscular contraction. Between the sensory and effector

parts of the nervous system is a message-processing (internuncial) part, whose complexity is not great in the jellyfish or the leech. However, the complexity of the internuncial part of the nervous system increases dramatically as one goes upward in the evolutionary order of animals, and in humans it is truly astonishing.

The small button-like connections between neurons are called synapses. When an electrical signal propagating along an axon reaches a synapse, it releases a chemical transmitter substance into the tiny volume between the synapse and the next neuron (the post-synaptic cleft). Depending on the nature of the synapse, this chemical messenger may either cause the next neuron to "fire" (i.e., to produce an electrical pulse along its axon) or it may inhibit the firing of the neuron. Furthermore, the question of whether a neuron will or will not fire depends on the past history of its synapses. Because of this feature, the internuncial part of an animal's nervous system is able to learn. There many kinds of synapses and many kinds of neurotransmitters, and the response of synapses is sensitive to the concentration of various molecules in the blood, a fact which helps to give the nervous systems of higher animals extraordinary subtlety and complexity.

The first known neurotransmitter molecule, acetylcholine, was discovered jointly by Sir Henry Dale in England and by Otto Loewi in Germany. In 1921 Loewi was able to show that nerve endings transmit information to muscles by means of this substance. The idea for the critical experiment occurred to him in a dream at 3 am. Otto Loewi woke up and wrote down the idea; but in the morning he could not read what he had written. Luckily he had the same dream the following night. This time he took no chances. He got up, drank some coffee, and spent the whole night working in his laboratory. By morning he had shown that nerve cells separated from the muscle of a frog's heart secrete a chemical substance when stimulated, and that this substance is able to cause contractions of the heart of another frog. Sir Henry Dale later showed that Otto Loewi's transmitter molecule was identical to acetylcholine, which Dale had isolated from the ergot fungus in 1910. The two men shared a Nobel Prize in 1936. Since that time, a large variety of neurotransmitter molecules have been isolated. Among the excitatory neurotransmitters (in addition to acetylcholine) are noradrenalin, norepinephrine, serotonin, dopamine, and glutamate, while gamma-amino-butyric acid is an example of an inhibitory neurotransmitter.

In 1953, Stephen W. Kuffler, working at Johns Hopkins University, made a series of discoveries which yielded much insight into the mechanisms by which the internuncial part of mammalian nervous systems processes information. Kuffler's studies showed that some degree of abstraction of patterns already takes place in the retina of the mammalian eye, before signals are passed on through the optic nerve to the visual cortex of the brain. In the mammalian retina, about 100 million light-sensitive primary light-receptor cells are connected through bipolar neurons to approximately a million retinal neurons of another type, called ganglions. Kuffler's first discovery (made using microelectrodes) was that even in total darkness, the retinal ganglions continue to fire steadily at the rate of about thirty pulses per second. He also found that diffuse light illuminating the entire retina does not change this steady rate of firing.

Kuffler's next discovery was that each ganglion is connected to an array of about 100 primary receptor cells, arranged in an inner circle surrounded by an outer ring. Kuffler

found the arrays to be of two types, which he called "on center arrays" and "off center arrays". In the "on center arrays", a tiny spot of light, illuminating only the inner circle, produces a burst of frequent firing of the associated ganglion, provided that cells in the outer ring of the array remain in darkness. However, if the cells in the outer ring are also illuminated, there is a cancellation, and there is no net effect. Exactly the opposite proved to be the case for the "off center arrays". As before, uniform illumination of both the inner circle and outer ring of these arrays produces a cancellation and hence no net effect on the steady background rate of ganglion firing. However, if the central circle by itself is illuminated by a tiny spot of light, the ganglion firing is inhibited, whereas if the outer ring alone is illuminated, the firing is enhanced. Thus Kuffler found that both types of arrays give no response to uniform illumination, and that both types of arrays measure, in different ways, the degree of contrast in the light falling on closely neighboring regions of the retina.

Kuffler's research was continued by his two associates, David H. Hubel and Torsten N. Wessel, at the Harvard Medical School, to which Kuffler had moved. In the late 1950's, they found that when the signals sent through the optic nerves reach the visual cortex of the brain, a further abstraction of patterns takes place through the arrangement of connections between two successive layers of neurons. Hubbel and Wessel called the cells in these two pattern-abstracting layers "simple" and "complex". The retinal ganglions were found to be connected to the "simple" neurons in such a way that a "simple" cell responds to a line of contrasting illumination of the retina. For such a cell to respond, the line has to be at a particular position and has to have a particular direction. However, the "complex" cells in the next layer were found to be connected to the "simple" cells in such a way that they respond to a line in a particular direction, even when it is displaced parallel to itself[2].

In analyzing their results, Kuffler, Hubel and Wessel concluded that pattern abstraction in the mammalian retina and visual cortex takes place through the selective destruction of information. This conclusion agrees with what we know in general about abstractions: They are always simpler than the thing which they represent.

6.3 The giant squid axon

The mechanism by which electrical impulses propagate along nerve ax- ons was clarified by the English physiologists Alan Lloyd Hodgkin and Andrew Fielding Huxley (a grandson of Darwin's defender, Thomas Henry Huxley). In 1952, working with the giant axon of the squid (which can be as large as a millimeter in diameter), they demonstrated that the electrical impulse propagating along a nerve is in no way similar to an electrical current in

[2] Interestingly, at about the same time, the English physiologist J.Z. Young came to closely analogous conclusions regarding the mechanism of pattern abstraction in the visual cortex of the octopus brain. However, the similarity between the image-forming eye of the octopus and the image-forming vertebrate eye and the rough similarity between the mechanisms for pattern abstraction in the two cases must both be regarded as instances of convergent evolution, since the mollusc eye and the vertebrate eye have evolved independently.

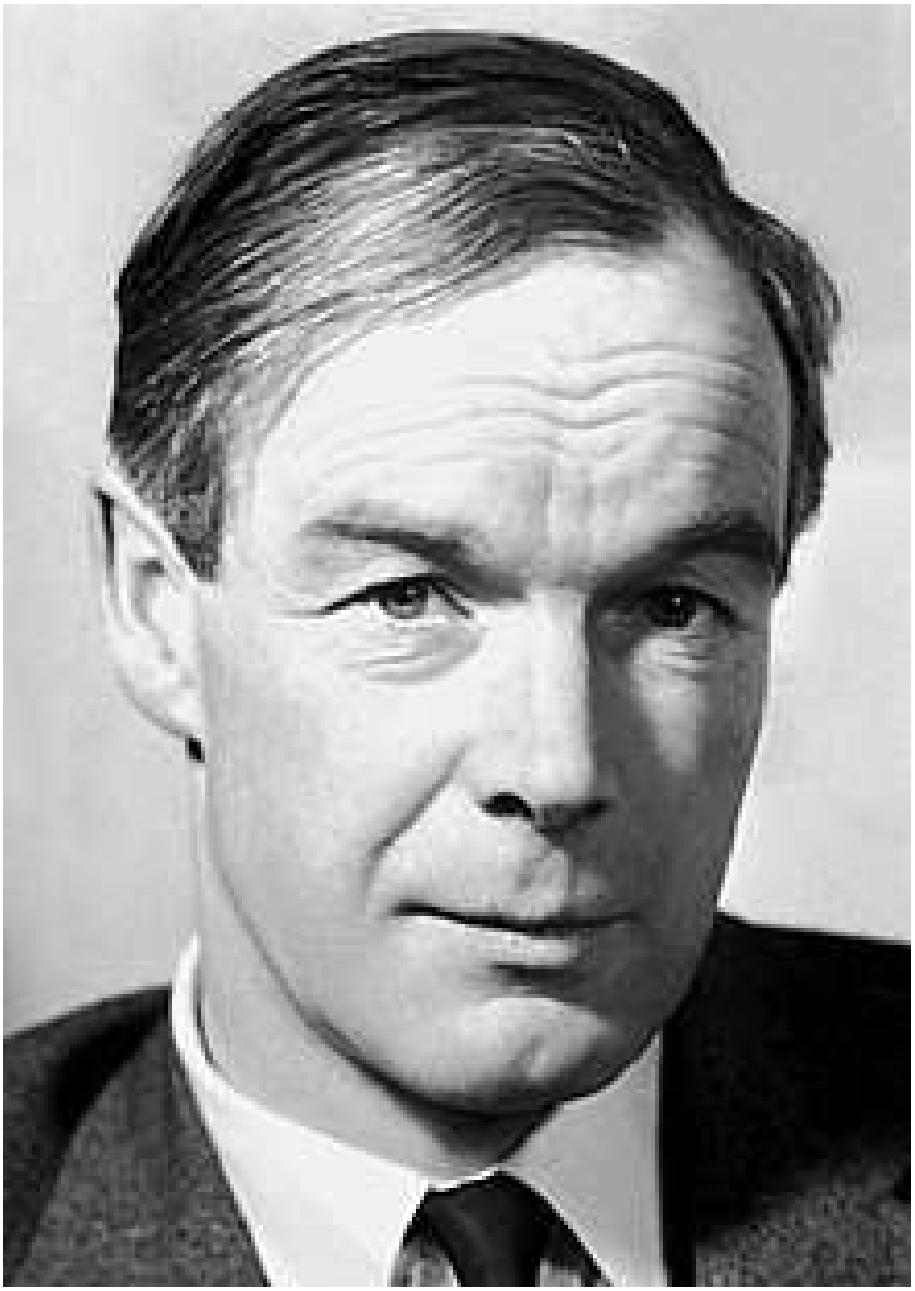

Figure 6.1: **Sir Alan Lloyd Hodgkin (1914-1998). He shared the 1963 Nobel Prize in Physiology or Medicine with Andrew Huxley and John Eccles.**

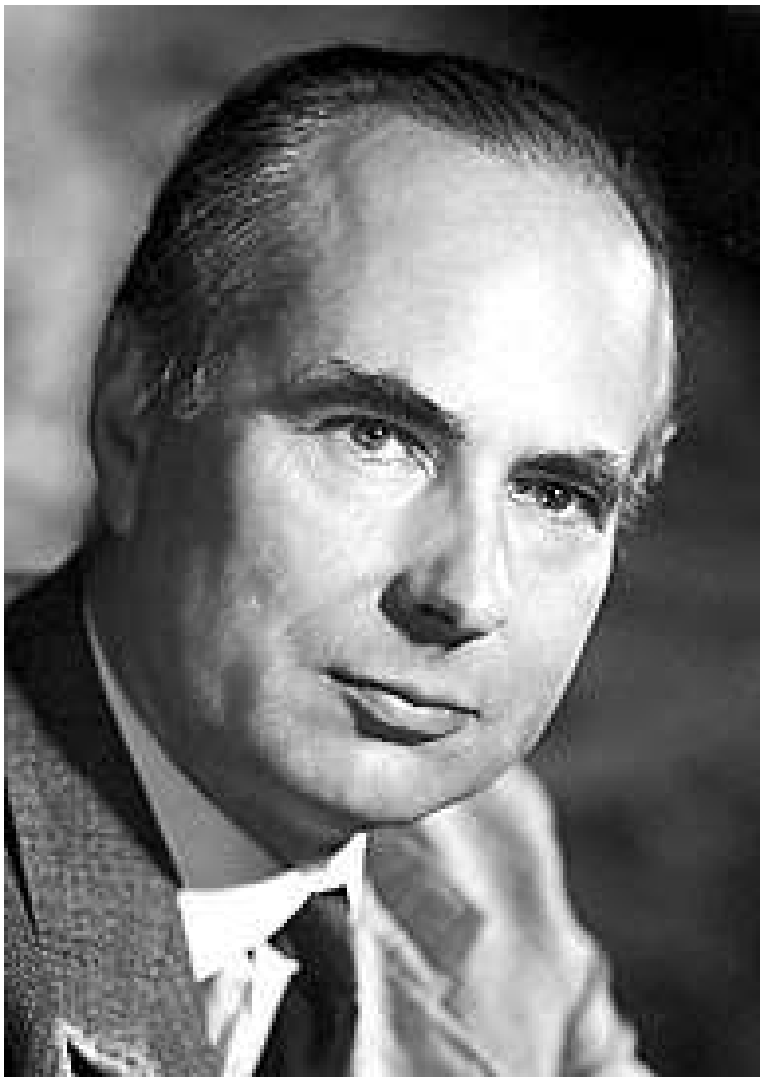

Figure 6.2: **Sir Andrew Fielding Huxley (1917-2012). He was a member of a famous family that included Thomas Henry Huxley ("Darwin's bulldog"), Aldous Huxley (author of *Brave New World*) and Sir Julian Huxley (a renowned evolutionary biologist, and the first director of UNESCO).**

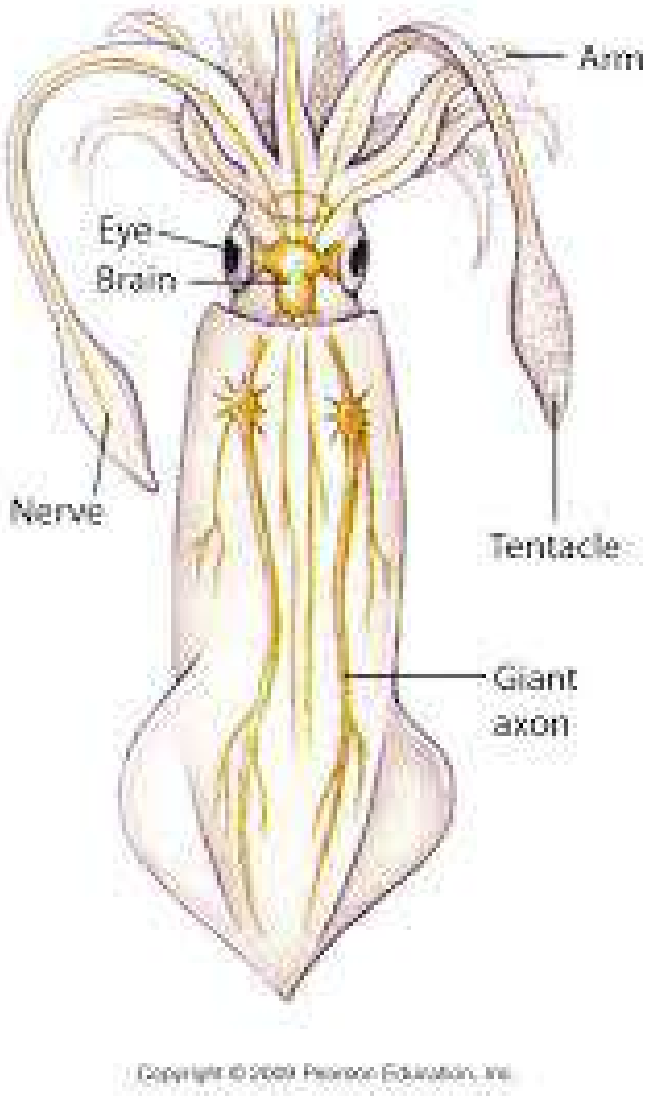

Figure 6.3: **The squid giant axon was large enough to allow Hodgkin and Huxley to perform their experiments demonstrating the mechanism of signal propagation in nerves. The squid giant axon was discovered by John Zachary Young (1907-1997) in the 1930's.**

Figure 6.4: **Hodgkin and Huxley working together.**

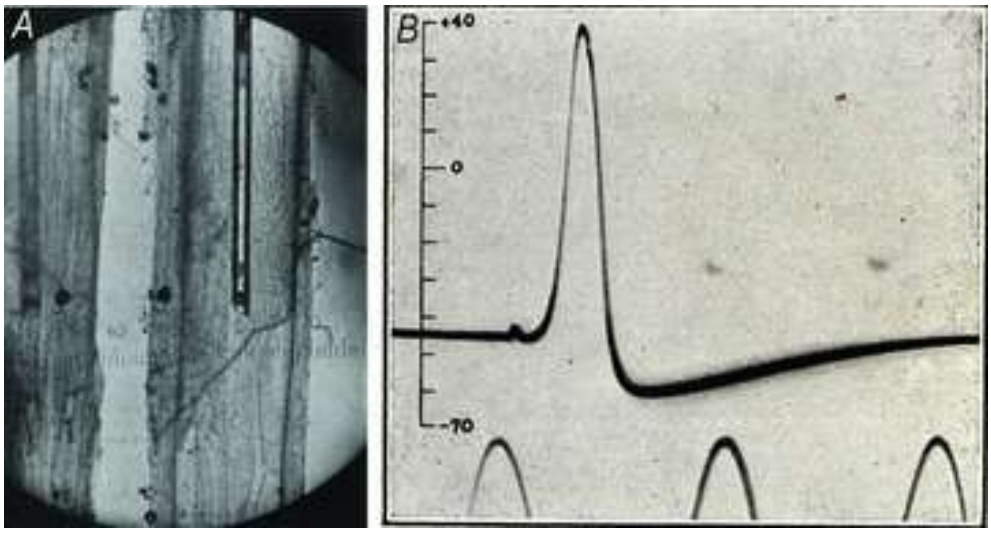

Figure 6.5: **Intracellular recording of the squid giant axon action potential.**

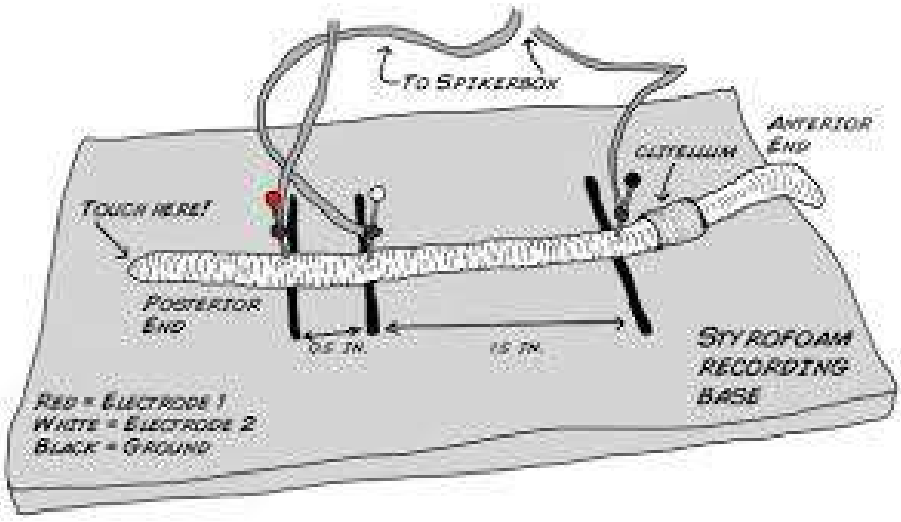

Figure 6.6: **A diagram of the Hodgkin-Huxley experiment with the giant squid axon.**

a conducting wire, but is more closely analogous to a row of dominoes knocking each other down. The nerve fiber, they showed, is like a long thin tube, within which there is a fluid containing K^+, and Na^+ ions, as well as anions. Inside a resting nerve, the concentration of K^+ is higher than in the normal body fluids outside, and the concentration of Na^+ is lower. These abnormal concentrations are maintained by an "ion pump", which uses the Gibbs free energy of adenosine triphosphate (ATP) to bring potassium ions into the nerve and to expel sodium ions.

The membrane surrounding the neural axon is more permeable to potassium ions than to sodium, and the positively charged potassium ions tend to leak out of the resting nerve, producing a small difference in potential between the inside and outside. This "resting potential" helps to hold the molecules of the membrane in an orderly layer, so that the membrane's permeability to ions is low.

Hodgkin and Huxley showed that when a neuron fires, the whole situation changes dramatically. Triggered by the effects of excitatory neurotransmitter molecules, sodium ions begin to flow into the axon, destroying the electrical potential which maintained order in the membrane. A wave of depolarization passes along the axon. Like a row of dominoes falling, the disturbance propagates from one section to the next: Sodium ions flow in, the order-maintaining electrical potential disappears, the next small section of the nerve membrane becomes permeable, and so on. Thus, Hodgkin and Huxley showed that when a neuron fires, a quick pulse-like electrical and chemical disturbance is transmitted along the axon.

Afterwards, the resting potential is restored by the sodium-potassium ion pump, later discovered by the Danish physiologist Jens Christian Skou. The pump consists of membrane-bound enzymes that use the energy of ATP to transport the ions across the electrochemical gradient.

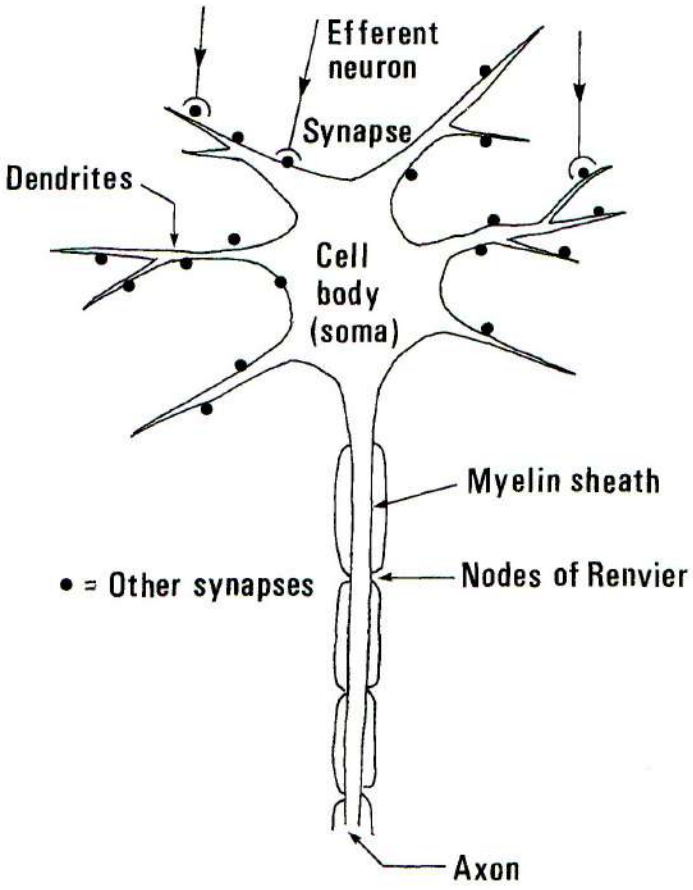

Figure 6.7: A schematic diagram of a neuron.

6.4 Chemical synapses

The small button-like connections between neurons are called synapses. When an electrical signal propagating along an axon reaches a synapse, it releases a chemical transmitter substance into the tiny volume between the synapse and the next neuron (the post-synaptic cleft). Depending on the nature of the synapse, this chemical messenger may either cause the next neuron to "fire" (i.e., to produce an electrical pulse along its axon) or it may inhibit the firing of the neuron. Furthermore, the question of whether a neuron will or will not fire depends on the past history of its synapses. Because of this feature, the internuncial part of an animal's nervous system is able to learn. There many kinds of synapses and many kinds of neurotransmitters, and the response of synapses is sensitive to the concentration of various molecules in the blood, a fact which helps to give the nervous systems of higher animals extraordinary subtlety and complexity.

6.5 Neurotransmitters

The first known neurotransmitter molecule, acetylcholine, was discovered jointly by Sir Henry Dale in England and by Otto Loewi in Germany. In 1921 Loewi was able to show that nerve endings transmit information to muscles by means of this substance.

The idea for the critical experiment occurred to him in a dream at 3 am. Otto Loewi woke up and wrote down the idea; but in the morning he could not read what he had written. Luckily he had the same dream the following night. This time he took no chances. He got up, drank some coffee, and spent the whole night working in his laboratory. By morning he had shown that nerve cells separated from the muscle of a frog's heart secrete a chemical substance when stimulated, and that this substance is able to cause contractions of the heart of another frog.

Sir Henry Dale later showed that Otto Loewi's transmitter molecule was identical to acetylcholine, which Dale had isolated from the ergot fungus in 1910. The two men shared a Nobel Prize in 1936. Since that time, a large variety of neurotransmitter molecules have been isolated. Among the excitatory neurotransmitters (in addition to acetylcholine) are noradrenalin, norepinephrine, serotonin, dopamine, and glutamate, while gamma-amino-butyric acid is an example of an inhibitory neurotransmitter.

Some important neurotransmitters

- **Glutamate**: This is the most abundant neurotransmitter in humans, used by about half of the neurons in the human brain. It is the primary excitatory transmitter in the central nervous system. One of its functions is to help form memories.

- **GABA**: The name GABA is an acronym for Gamma-aminobutyric acid. GABA is the primary inhibitory transmitter in the vertebrate brain. It helps to control anxiety, and it is sometimes used medically to treat anxiety and the associated sleeplessness.

- **Glycine**: This neurotransmitter is a single amino acid. It is the main inhibitory neurotransmitter in the vertebrate spinal cord. Glycine is important in the central nervous system, especially in the spinal cord, brainstem, and retina.

- **Acetylcholine**: An ester (the organic analogue of a salt) formed from the reaction between choline and acetic acid, acetylcholine stimulates muscles, functions in the autonomic nervous system and sensory neurons, and is associated with REM sleep. Alzheimer's disease is associated with a significant drop in acetylcholine levels.

- **Norepinepherine**: Also known as noradrenaline, norepinephorine increases heart rate and blood pressure. It is part of the body's "fight or flight" system. Norepinephrine is also needed to form memories. Stress depletes stores of this neurotransmitter.

- **Dopamine**: Dopamine is also synthesized in plants and most animals. It is an inhibitory transmitter associated with the reward center of the brain. Low dopamine levels are associated with social anxiety and Parkinson's disease, while excess dopamine is related to schizophrenia. The brain includes several distinct dopamine pathways, one of which plays a major role in reward-motivated behavior. Most types of rewards increase the level of dopamine in the brain, and many addictive drugs increase dopamine neuronal activity.

- **Serotonin**: Biochemically derived from the amino acid tryptophanis, serotonin an inhibitory neurotransmitter involved in mood, emotion, and perception. Low serotonin levels can lead to depression, suicidal tendencies, anger management issues, difficulty sleeping, migraines, and an increased craving for carbohydrates. It's functions include the regulation of mood, appetite, and sleep. Serotonin also has some cognitive functions, including memory and learning.

- **Endorphins**: The name of this class of neurotransmitters means "a class of a morphine-like substance originating from within the body". are a class of molecules similar to opioids (e.g., morphine, heroin) in terms of structure and function. The word "endorphin" is short for "endogenous morphine." Endorphins are inhibitory transmitters associated with pleasure and pain relief. In other animals, these chemicals slow metabolism and permit hibernation. The treatment of pain by means of acupuncture functions by releasing endorphines.

6.6 Transmission of signals across synapses

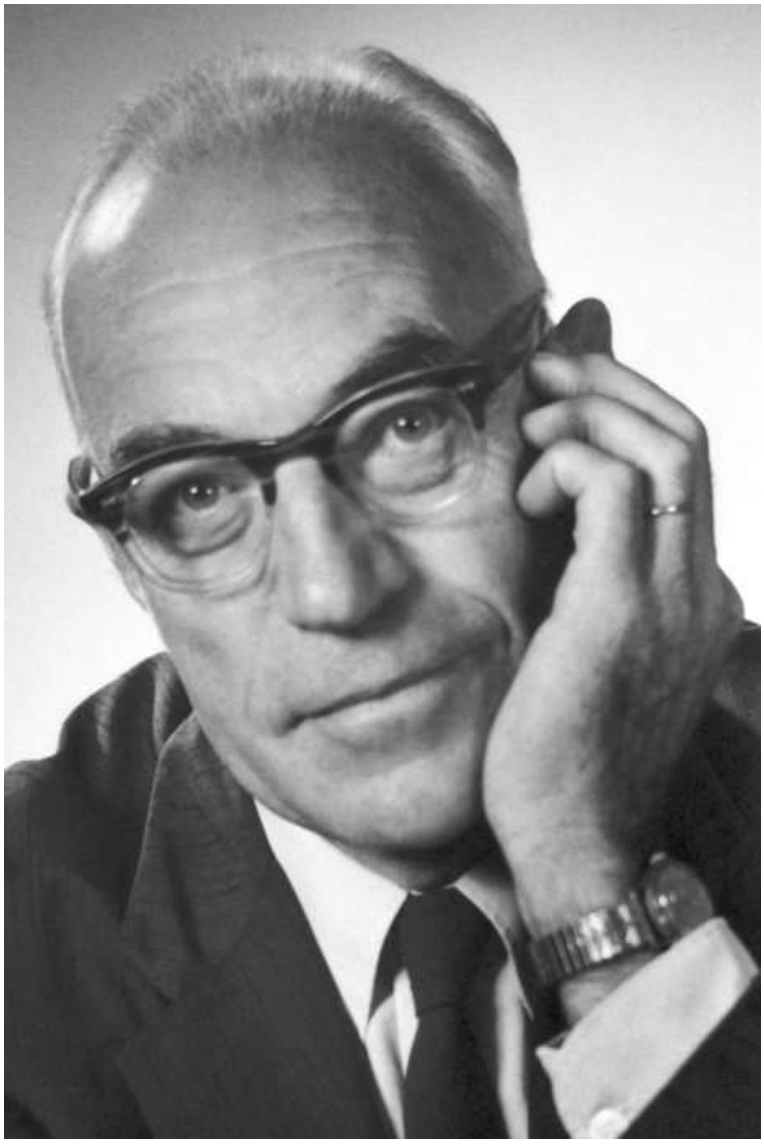

Figure 6.8: **Sir John Carew Eccles (1903-1997).**

Figure 6.9: **Jens Christian Skou (1908-2018). He received a Nobel Prize in Chemistry in in 1997 for his discovery of the K^+-Na^+ ion pump that uses energy from ATP to transport the ions across membranes against the electrochemical gradient. The photo shows him in 2008. He was born in Lemvig, Denmark.**

6.7 Are matter and mind separate?

One could, in principle, supply a computer with an input stream of sensory data, and program the computer to perform actions on the external world. In fact, the computer could be programmed in such a way that the actions taken would depend on the stored memory of previous sensory input. Could the computer then be said to be conscious? This depends on the way in which we define the word "conscious", and so the question is a semantic one, depending on our choice of a definition.

In any case, such a computer arrangement would be very closely analogous to the way in which living organisms experience their environment and act on it. Even the most primitive organisms receive a continuous stream of input data, and, if we choose, we can call this stream an elementary form of consciousness. Living organisms then react to the input stream, and their reactions may be modified by stored information of previous input data. The modification of response on the basis of previous experience is usually called "internuncial" modification, and it will be discussed below.

The pioneering Estonian scientist Jakob von Uexküll, whom we will discuss in detail below, introduced the word "Umwelt", which he defined to be the stream of sensory input data experienced by an organism. For example, speaking of a tick, he wrote: "...this eyeless animal finds the way to her watchpoint [at the top of a tall blade of grass] with the help of only its skin's general sensitivity to light. The approach of her prey becomes apparent to this blind and deaf bandit only through her sense of smell. The odor of butyric acid, which emanates from the sebaceous follicles of all mammals, works on the tick as a signal that causes her to abandon her post (on top of the blade of grass/bush) and fall blindly downward toward her prey. If she is fortunate enough to fall on something warm (which she perceives by means of an organ sensible to a precise temperature) then she has attained her prey, the warm-blooded animal, and thereafter needs only the help of her sense of touch to find the least hairy spot possible and embed herself up to her head..."

Figure 6.10: **The French philosopher, mathematician and scientist René Descartes (1596-1650) advocated mind-matter dualism. Descartes thought that nerves bring sensory inputs to the brain, where the data are then transferred to the "soul". After some time, he thought, the soul tells the brain how how the human should respond. Descartes did not discuss the question of whether organisms very low on the evolutionary scale have souls. Darwin visualized a continuous evolutionary progression from lower forms of life to ourselves. At what point did these less developed organisms obtain souls? Everyone must find his or her own opinion on this question.**

6.8 Jakob von Uexküll and Umwelt

Jakob Johann, Baron von Uexküll (1864-1944) was born in Estonia, on the estate of his aristocratic parents, Alexander, Baron von Uexküll and Sophie von Hahn. The family lost most of their wealth by expropriation during the Russian Revolution, and Jakob was forced to earn a living. He studied zoology at the University of Tartu. After graduation, he worked at the Institute of Physiology at the University of Heidelberg, and later at the Zoological Station in Naples. In 1907, he was given an honorary doctorate by Heidelberg for his studies of the physiology of muscles. Among his discoveries in this field was the first recognized instance of negative feedback in an organism.

Later work was concerned with the way in which animals experience the world around them. To describe the animal's subjective perception of its environment he introduced the word Umwelt; and in 1926 he founded the Institut für Umweltforschung at the University of Hamburg. Von Uexküll visualized an animal - for example a mouse - as being surrounded by a world of its own - the world conveyed by its own special senses organs, and processed by its own interpretative systems. Obviously, the Umwelt will differ greatly depending on the organism. For example, bees are able to see polarized light and ultraviolet light; electric eels are able to sense their environment through their electric organs; many insects are extraordinarily sensitive to pheromones; and a dog's Umwelt far richer in smells than that of most other animals. The Umwelt of a jellyfish is very simple, but nevertheless it exists.

It is interesting to ask to what extent the concept of Umwelt can be equated to that of consciousness. To the extent that these two concepts can be equated, von Uexküll's Umweltforschung offers us the opportunity to explore the phylogenetic evolution of the phenomenon of consciousness.

Von Uexküll's Umwelt concept can even extend to one-celled organisms, which receive chemical and tactile signals from their environment, and which are often sensitive to light. The ideas and research of Jakob von Uexküll inspired the later work of the Nobel Laureate ethologist Konrad Lorenz, and thus von Uexküll can be thought of as one of the founders of ethology as well as of biosemiotics. Indeed, ethology and biosemiotics are closely related. Because of his work on feedback loops in living organisms, von Uexküll can also be thought of as an early pioneer of cybernetics. His work influenced the philosophers Max Scheler, Ernst Cassirer, Martin Heidegger, Maurice Merleau-Ponty, Humberto Maturana, Georges Canguilhem, Michel Foucault, Gilles Deleuze and Félix Guattari.

Interestingly, his grandson, Carl Wolmar Jakob, Baron von Uexküll (born 1944) became a member of the European Parliament and contributed the funds for the Right Livelihood Award, which has been called the "Alternative Nobel Prize". Carl Wolmer Jakob is also the co-founder of the World Future Council and the Other Economic Summit.

Amoebae, slime molds and sponges

Amoebae are eukaryotes that have the ability to alter their shape. Like other eukaryotes they have a cell nucleus and other organelles, such as mitochondria, surrounded by an

Figure 6.11: **Jakob Johann, Baron von Uexküll (1864-1944) was the founder of Umwelt research. He was also an early pioneer of Cybernetics and Biosemiotics.**

Figure 6.12: **Carl Wolmar Jakob, Baron von Uexküll (born 1944) co-founded the World Future Council and the Other Economic Summit, as well as contributing the money needed to fund the Right Livelihood Award.**

Figure 6.13: **The Copenhagen-Tartu school of biosemiotics is a network of scholars working in the field of biosemiotics at the University of Tartu and the University of Copenhagen. An important member of the group is Center Leader Claus Emmeche of the Niels Bohr Institute (shown here). Other members include Kalevi Kull, Jesper Hoffmeyer, Peeter Torop, Timo Maran and Mikhail Lotman.**

outer membrane. Amoebae often eat bacteria by engulfing them.

More than 900 species of slime molds exist in various parts of the world. They are very common on the floors of tropical rain forests, where they perform the valuable service of helping to recycle nutrients.

Slime molds are particularly interesting because they gives us a glimpse of how multicellular organisms may have originated. The name of the slime molds is misleading, since they are not fungi, but heterotrophic protists similar to amoebae. Under ordinary circumstances, the individual cells wander about independently searching for food, which they draw into their interiors and digest, a process called "phagocytosis". However, when food is scarce, they send out a chemical signal of distress. Researchers have analyzed the molecule which expresses slime mold unhappiness, and they have found it to be cyclic adenosine monophosphate (cAMP). At this signal, the cells congregate and the mass of cells begins to crawl, leaving a slimy trail. At it crawls, the community of cells gradually develops into a tall stalk, surmounted by a sphere - the "fruiting body". Inside the sphere, spores are produced by a sexual process. If a small animal, for example a mouse, passes by, the spores may adhere to its coat; and in this way they may be transported to another part of the forest where food is more plentiful.

Thus slime molds represent a sort of missing link between unicellular and multicellular or organisms. Normally the cells behave as individualists, wandering about independently, but when challenged by a shortage of food, the slime mold cells join together into an entity which closely resembles a multicellular organism. The cells even seem to exhibit altruism, since those forming the stalk have little chance of survival, and yet they are willing to perform their duty, holding up the sphere at the top so that the spores will survive and carry the genes of the community into the future. We should especially notice the fact that the cooperative behavior of the slime mold cells is coordinated by chemical signals.

Sponges are also close to the borderline which separates unicellular eukaryotes (protists) from multicellular organisms, but they are just on the other side of the border. Normally the sponge cells live together in a multicellular community, filtering food from water. However, if a living sponge is forced through a very fine cloth, it is possible to separate the cells from each other. The sponge cells can live independently for some time; but if many of them are left near to one another, they gradually join together and form themselves into a new sponge, guided by chemical signals. In a refinement of this experiment, one can take two living sponges of different species, separate the cells by passing the sponges through a fine cloth, and afterwards mix all the separated cells together. What happens next is amazing: The two types of sponge cells sort themselves out and become organized once more into two sponges - one of each species.

Slime molds and sponges hint at the genesis of multicellular organisms, whose evolution began approximately 600 million years ago. Looking at the slime molds and sponges, we can imagine how it happened. Some unicellular organisms must have experienced an enhanced probability of survival when they lived as colonies. Cooperative behavior and division of labor within the colonies were rewarded by the forces of natural selection, with the selective force acting on the entire colony of cells, rather than on the individual cell. This resulted in the formation of cellular societies and the evolution of mechanisms for cell

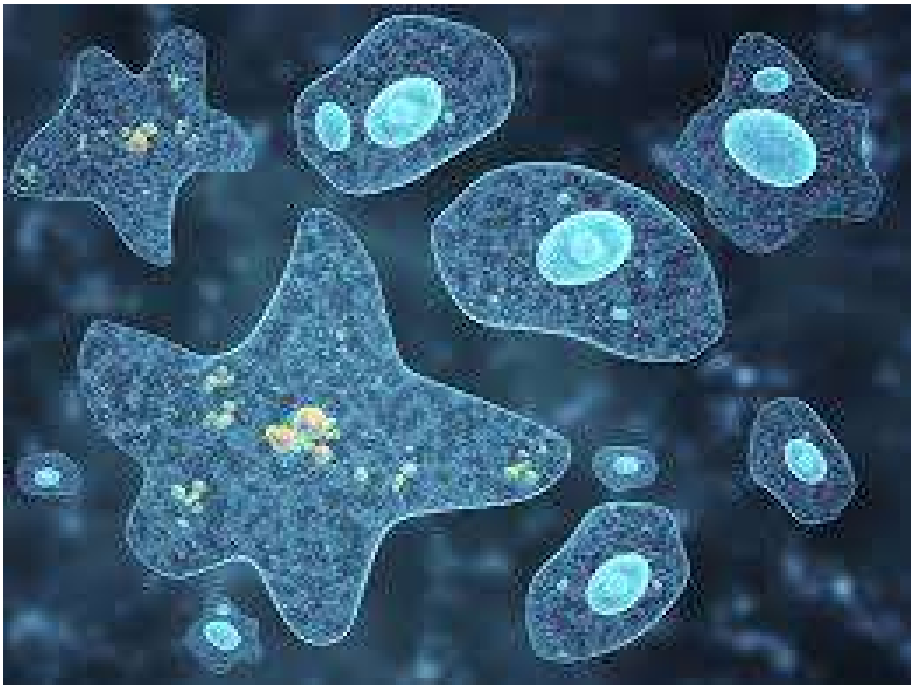

Figure 6.14: **Amoebae are eukaryotes, with a nucleus and other organelles, such as mitochondria, contained within a cell membrane. They are able to change their shapes, and often eat bacteria by engulfing them.**

differentiation. The division of labor within cellular societies (i.e., differentiation) came to be coordinated by chemical signals which affected the transcription of genetic information and the synthesis of proteins. Each cell within a society of cells possessed the entire genome characteristic of the colony, but once a cell had been assigned its specific role in the economy of the society, part of the information became blocked - that is, it was not expressed in the function of that particular cell. As multicellular organisms evolved, the chemical language of intercellular communication became very much more complex and refined. later section.

The world as seen by a jellyfish

Not all jellyfish are alike. Some species have much more highly-developed sensory perception than others. Jellyfish can swim, and their motions are coordinated by a rudimentary nervous system.

According to Wikipedia, "Jellyfish employ a loose network of nerves, located in the epidermis, which is called a 'nerve net'. Although traditionally thought not to have a central nervous system, nerve net concentration and ganglion-like structures could be considered to constitute one in most species. A jellyfish detects various stimuli including the touch of other animals via this nerve net, which then transmits impulses both throughout the nerve net and around a circular nerve ring, through the rhopalial lappet, located at the rim of

Figure 6.15: **The fruiting bodies of a slime mold.**

Figure 6.16: **Like slime molds, sponges are close to the borderline between single-celled and multi-cellular organisms.**

Figure 6.17: **How does a jellyfish experience the world around it?**

the jellyfish body, to other nerve cells.

"Some jellyfish have ocelli: light-sensitive organs that do not form images but which can detect light and are used to determine up from down, responding to sunlight shining on the water's surface. These are generally pigment spot ocelli, which have some cells (not all) pigmented.

"Certain species of jellyfish, such as the box jellyfish, have more advanced vision than their counterparts. The box jellyfish has 24 eyes, two of which are capable of seeing color, and four parallel information processing areas or rhopalia that act in competition, supposedly making it one of the few creatures to have a 360-degree view of its environment.

"The eyes are suspended on stalks with heavy crystals on one end, acting like a gyroscope to orient the eyes skyward. They look upward to navigate from roots in mangrove swamps to the open lagoon and back, watching for the mangrove canopy, where they feed."

6.9 Biosemiotics

The Oxford Dictionary of Biochemistry and Molecular Biology (Oxford University Press, 1997) defines biosemiotics as "the study of signs, of communication, and of information in living organisms". The biologists Claus Emmeche and K. Kull offer another definition of biosemiotics: "biology that interprets living systems as sign systems".

The American philosopher Charles Sanders Peirce (1839-1914) is considered to be one of the founders of semiotics (and hence also of biosemiotics). Peirce studied philosophy and

chemistry at Harvard, where his father was a professor of mathematics and astronomy. He wrote extensively on philosophical subjects, and developed a theory of signs and meaning which anticipated many of the principles of modern semiotics. Peirce built his theory on a triad: (1) the sign, which represents (2) something to (3) somebody. For example, the sign might be a broken stick, which represents a trail to a hunter, it might be the arched back of a cat, which represents an aggressive attitude to another cat, it might be the waggle-dance of a honey bee, which represents the coordinates of a source of food to her hive-mates, or it might be a molecule of trans-10-cis-hexadecadienol, which represents irresistible sexual temptation to a male moth of the species Bombyx mori. The sign might be a sequence of nucleotide bases which represents an amino acid to the ribosome-transfer-RNA system, or it might be a cell-surface antigen which represents self or non-self to the immune system. In information technology, the sign might be the presence or absence of a pulse of voltage, which represents a binary digit to a computer. Semiotics draws our attention to the sign and to its function, and places much less emphasis on the physical object which forms the sign. This characteristic of the semiotic viewpoint has been expressed by the Danish biologist Jesper Hoffmeyer in the following words: "The sign, rather than the molecule, is the basic unit for studying life."

A second important founder of biosemiotics was Jakob von Uexküll (1864-1944). He was born in Estonia, and studied zoology at the University of Tartu. After graduation, he worked at the Institute of Physiology at the University of Heidelberg, and later at the Zoological Station in Naples. In 1907, he was given an honorary doctorate by Heidelberg for his studies of the physiology of muscles. Among his discoveries in this field was the first recognized instance of negative feedback in an organism. Von Uexküll's later work was concerned with the way in which animals experience the world around them. To describe the animal's subjective perception of its environment he introduced the word Umwelt; and in 1926 he founded the Institut fur Umweltforschung at the University of Heidelberg. Von Uexküll visualized an animal - for example a mouse - as being surrounded by a world of its own - the world conveyed by its own special senses organs, and processed by its own interpretative systems. Obviously, the Umwelt will differ greatly depending on the organism. For example, bees are able to see polarized light and ultraviolet light; electric eels are able to sense their environment through their electric organs; many insects are extraordinarily sensitive to pheromones; and a dog's Umwelt far richer in smells than that of most other animals. The Umwelt of a jellyfish is very simple, but nevertheless it exists.[3] Von Uexküll's Umwelt concept can even extend to one-celled organisms, which receive chemical and tactile signals from their environment, and which are often sensitive to light. The ideas and research of Jakob von Uexk"ull inspired the later work of the Nobel Laureate ethologist Konrad Lorenz, and thus von Uexküll can be thought of as one of the founders of ethology as well as of biosemiotics. Indeed, ethology and biosemiotics are closely related.

Biosemiotics also values the ideas of the American anthropologist Gregory Bateson

[3] It is interesting to ask to what extent the concept of Umwelt can be equated to that of consciousness. To the extent that these two concepts can be equated, von Uexküll's Umweltforschung offers us the opportunity to explore the phylogenetic evolution of the phenomenon of consciousness.

(1904-1980), who was mentioned in Chapter 7 in connection with cybernetics and with the Macy Conferences. He was married to another celebrated anthropologist, Margaret Mead, and together they applied Norbert Wiener's insights concerning feedback mechanisms to sociology, psychology and anthropology. Bateson was the originator of a famous epigrammatic definition of information: "..a difference which makes a difference" . This definition occurs in Chapter 3 of Bateson's book, Mind and Nature: A Necessary Unity, Bantam, (1980), and its context is as follows: "To produce news of a difference, i.e. information", Bateson wrote, "there must be two entities... such that news of their difference can be represented as a difference inside some information-processing entity, such as a brain or, perhaps, a computer. There is a profound and unanswerable question about the nature of these two entities that between them generate the difference which becomes information by making a difference. Clearly each alone is - for the mind and perception - a non-entity, a non-being... the sound of one hand clapping. The stuff of sensation, then, is a pair of values of some variable, presented over time to a sense organ, whose response depends on the ratio between the members of the pair."

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Chapter 7

WATER AND BIOLOGICAL SPECIFICITY

7.1 Hydrogen bonds in water

In the water molecule, there is a small positive excess charge, $+\delta$, on each of the hydrogens, and a small negative excess charge, -2δ, on the oxygen. Hydrogen bonds in water and ice are formed by Coulomb attractions between these positive and negative charges. In the figure shown below, the hydrogen bonds are represented by dotted lines. The insolubility of nonpolar molecules is due to the fact that they break up the hydrogen bonds in water, and it thus costs energy to incorporate them into water.

Polar molecules, on the other hand, can fit into the hydrogen bonding system of water by forming their own hydrogen bonds with water molecules, and thus they are water-soluble.

Soaps and detergents have a polar end, attached to a long nonpolar tail. They allow groups of nonpolar molecules to become water-soluble by forming a layer with the polar ends pointing outward to the water, while the long non-polar ends point inwards.

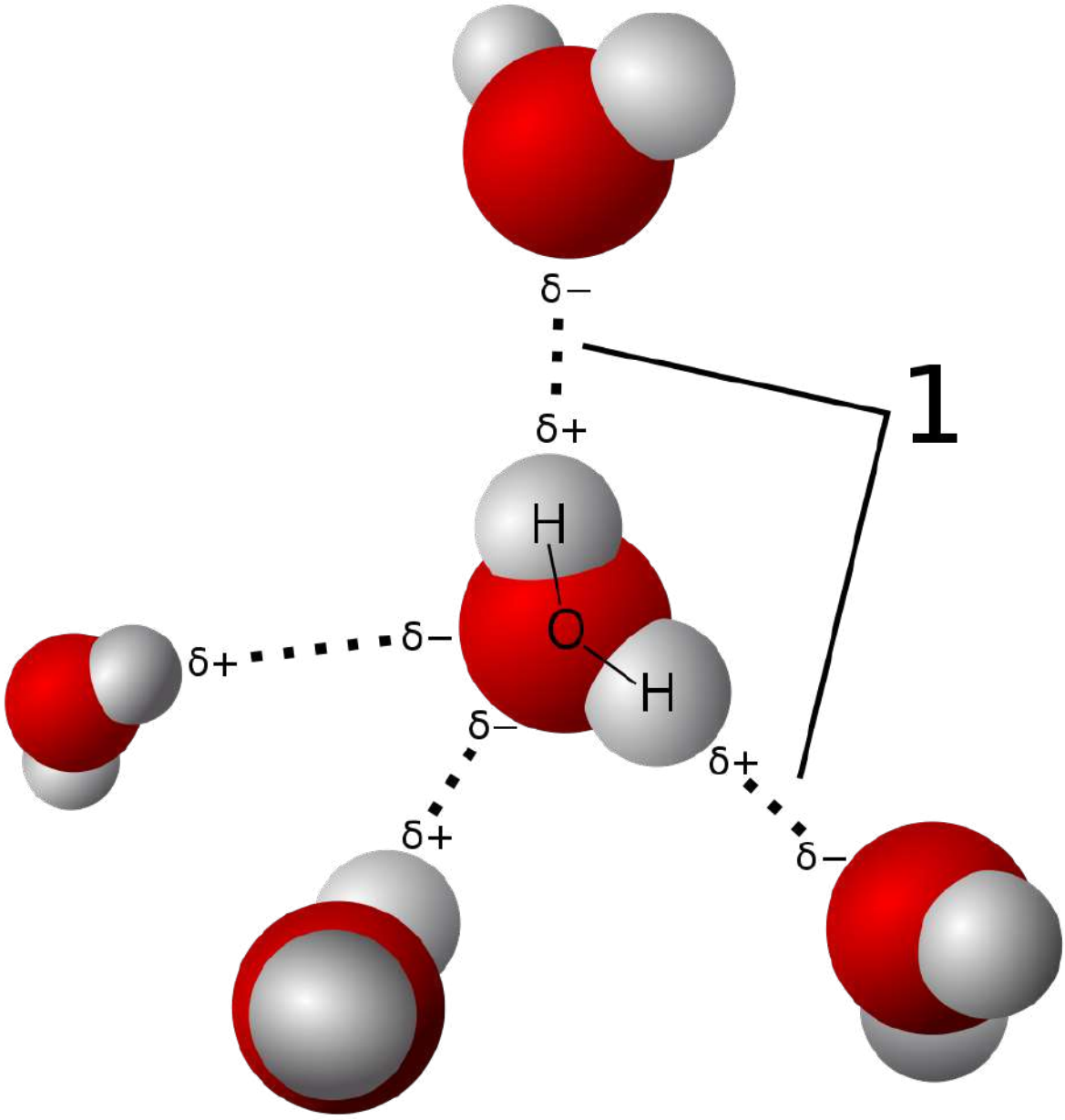

Figure 7.1: **In the water molecule, there is a small positive excess charge, $+\delta$, on each of the hydrogens, and a small negative excess charge, -2δ, on the oxygen. Hydrogen bonds in water and ice are formed by Coulomb attractions between these positive and negative charges. In this figure, the hydrogen bonds are represented by dotted lines. The insolubility of nonpolar molecules is due to the fact that they break up the hydrogen bonds in water, and it thus costs energy to incorporate them into water.**

7.2 Water and the folding of proteins

When I worked at the Imperial College of Science and Technology in London, during the 1960's, I was a member of the Royal Institution of Great Britain, where Michael Faraday was once the director, and where Faraday gave lectures on science that were attended by Queen Victoria's husband, Prince Albert and his sons.

The tradition of polished and entertaining lectures initiated by Faraday is continued today. I vividly remember attending a lecture on the structure of the protein, lysozyme.

Lysozyme was the first antibacterial agent discovered by Alexander Fleming. He was disappointed to find that the pathogenic bacteria against which it is effective are not associated with very serious diseases. In fact, these diseases are not serious because the human body produces the enzyme lysozyme. We have it, for example, in our nasal mucus.

But back to the Royal Institution lecture on the structure of lysozyme, which had been determined by the use of X-ray crystallography. As in Faraday's day, the lecture was given with much style. The lecturer was the person responsible for solving the structure, David Chilton Phillips (1925-1999), who was later made a Life Peer, Baron Phillips of Ellesmere.

Hanging from the ceiling of the lecture room was a long chain model of the amino acid sequence of the lysozyme macro-molecule, before folding. D.C. Phillips explained all the difficulties of obtaining good crystals and performing the X-ray diffraction experiments. Then he said "Finally, after much work, and a little prayer, we obtained a structure", and he gazed upward, as if to heaven. Then dramatically, a model of the folded protein was lowered downward towards us from its previously unseen position at the top of the room.

Phillips flipped a switch, and we saw on the linear model, the positions of the hydrophilic amino acids and the hydrophobic ones, indicated respectively by green and red lights, Then flipping another switch, he showed us their positions on the folded molecule. The hydrophilic amino acids were all on the outside, while the hydrophobic ones were on the inside. The surrounding water had determined the way in which the protein had folded (its tertiary structure) as well as its enzymatic activity. We could see clearly the active site of lysozyme, its "mouth", where it bit into the cell walls of bacteria.

The case of lysozyme is surely not an isolated one. It seems logical to generalize from this case, and to think that the tertiary structure and enzymatic activity of all water-soluble proteins is determined by the interaction of hydrophilic and hydrophobic amino acids with the surrounding water.

7.3 The second law of thermodynamics

The second law of thermodynamics was discovered by Nicolas Leonard Sadi Carnot (1796-1832) and elaborated by Rudolf Clausius (1822-1888) and William Thomson (later Lord Kelvin, 1824-1907). Carnot came from a family of distinguished French politicians and military men, but instead of following a political career, he studied engineering. In 1824,

his only scientific publication appeared - a book with the title *Reflections on the Motive Power of Fire*. Although it was ignored for the first few years after its publication, this single book was enough to secure Carnot a place in history as the founder of the science of thermodynamics. In his book, Carnot introduced a scientific definition of work which we still use today - "weight lifted through a height"; in other words, force times distance.

At the time when Carnot was writing, much attention was being given to improving the efficiency of steam engines. Although James Watt's steam engines were far more efficient than previous models, they still could only convert between 5 % and 7 % of the heat energy of their fuels into useful work. Carnot tried to calculate the theoretical maximum of the efficiency of steam engines, and he was able to show that an engine operating between the temperatures T_1 and T_2 could at most attain

$$\text{maximum efficiency} = \frac{T_1 - T_2}{T_1} \tag{7.1}$$

Here T_1 is the temperature of the input steam, and T_2 is the temperature of the cooling water. Both these temperatures are absolute temperatures, i.e., temperatures proportional to the volume of a given quantity of gas at constant pressure.

Carnot died of cholera at the age of 36. Fifteen years after his death, the concept of absolute temperature was further clarified by Lord Kelvin (1824-1907), who also helped to bring Carnot's work to the attention of the scientific community.

Building on the work of Carnot, the German theoretical physicist Rudolph Clausius was able to deduce an extremely general law. He discovered that the ratio of the heat content of a closed system to its absolute temperature always increases in any process. He called this ratio the entropy of the system. In the notation of modern thermodynamics, the change in entropy dS when a small amount of heat dq is transferred to a system is given by

$$dS = \frac{dq}{dT} \tag{7.2}$$

Let us imagine a closed system consisting of two parts, one at temperature T_1, and the other part at a lower temperature T_2. If a small amount of heat dq flows from the warmer part to the cooler one, the small resulting change in entropy of the total system will be

$$dS = \frac{dq}{T_1} - \frac{dq}{T_2} > 0 \tag{7.3}$$

According to Clausius, since heat never flows spontaneously from a colder object to a warmer one, the entropy of a closed system always increases; that is to say, dS is always positive. As heat continues to flow from the warmer part of the system to the cooler part, the system's energy becomes less and less available for doing work. Finally, when the two parts have reached the same temperature, no work can be obtained. When the parts differed in temperature, a heat engine could in principle be run between them, making use of the temperature difference; but when the two parts have reached the same temperature, this possibility no longer exists. The law stating that the entropy of a closed system always increases is called the second law of thermodynamics.

7.4 Statistical mechanics

Besides his monumental contributions to electromagnetic theory, the English physicist James Clerk Maxwell (1831-1879) also helped to lay the foundations of statistical mechanics. In this enterprise, he was joined by the Austrian physicist Ludwig Boltzmann (1844-1906) and by an American, Josiah Willard Gibbs, whom we will discuss later.

As a young student, Boltzmann read Maxwell's paper on the velocity distributions of molecules in a gas, and he spent the remainder of his life developing these Maxwell's initiative into the science of statistical mechanics. Boltzmann was able to derive the following equation hold for the particles in a perfect (non-interacting) gas:

$$\frac{n_i}{N} = \frac{e^{-\epsilon_i/kT}}{\sum_i e^{-\epsilon_i/kT}} \tag{7.4}$$

Here n_i represents the number of particles in a state with energy ϵ_l, while N is the total number of particles. T is the absolute temperature, and k, which is called *Boltzmann's constant*, has a dimension such that the dimension of kT is energy.

Like Maxwell, Boltzmann also interpreted an increase in entropy as an increase in disorder; and like Maxwell he was a firm believer in atomism at a time when this belief was by no means universal. For example, Ostwald and Mach, both important figure in German science at that time, refused to believe in the existence of atoms, in spite of the fact that Dalton's atomic ideas had proved to be so useful in chemistry. Towards the end of his life, Boltzmann suffered from periods of severe depression, perhaps because of attacks on his scientific work by Ostwald and others. In 1906, while on vacation near Trieste, he committed suicide - ironically, just a year before the French physicist J.B. Perrin produced irrefutable evidence of the existence of atoms.

When a system is in thermodynamic equilibrium, its entropy has reached a maximum; but if it is not in equilibrium, its entropy has a lower value. For example, let us think of the case which was studied by Clausius when he introduced the concept of entropy: Clausius imagined an isolated system, divided into two parts, one of which has a temperature T_i, and the other a lower temperature, T_2. When heat is transferred from the hot part to the cold part, the entropy of the system increases; and when equilibrium is finally established at some uniform intermediate temperature, the entropy has reached a maximum. The difference in entropy between the initial state of Clausius' system and its final state is a measure of how far away from thermodynamic equilibrium it was initially.

Figure 7.2: **The English physicist James Clerk Maxwell (1931-1879). Together with Ludwig Boltzmann, he was one of the founders of statistical mechanics. Maxwell took the first step in a paper on the velocity distributions of molecules in a gas.**

Figure 7.3: **The Austrian physicist Ludwig Boltzmann (1844-1906), the co-founder of statistical mechanics. As a young student, Boltzmann read Maxwell's paper on velocity distributions, and he spent the remainder of his life developing these ideas into the science of statistical mechanics.**

7.5 Gibbs free energy

The American physicist Josiah Willard Gibbs (1839-1903) made many contributions to thermodynamics and statistical mechanics. In 1863, Gibbs received from Yale the first Ph.D. in engineering granted in America, and after a period of further study in France and Germany, he became a professor of mathematical physics at Yale in 1871, a position which he held as long as he lived. During the period between 1876 and 1878, he published a series of papers in the *Transactions of the Connecticut Academy of Sciences*. In these papers, about 400 pages in all, Gibbs applied thermodynamics to chemical reactions. (The editors of the *Transactions of the Connecticut Academy of Sciences* did not really understand Gibbs' work, but, as they said later, "We knew Gibbs, and we took his papers on faith".)

Because the journal was an obscure one, and because Gibbs' work was so highly mathematical, it remained almost unknown to European scientists for a long period. However, in 1892 Gibbs' papers were translated into German by Ostwald, and in 1899 they were translated into French by Le Chatelier; and then the magnitude of Gibbs' contribution was finally recognized. One of his most important innovations was the definition of a quantity which we now call "Gibbs free energy". This quantity allows one to determine whether or not a chemical reaction will take place spontaneously.

Chemical reactions usually take place at constant pressure and constant temperature. If a reaction produces a gas as one of its products, the gas must push against the pressure of the earth's atmosphere to make a place for itself. In order to take into account the work done against external pressure in energy relationships, the German physiologist and physicist Hermann von Helmholtz introduced a quantity (which we now call heat content or enthalpy) defined by

$$H = U + PV \tag{7.5}$$

where U is the internal energy of a system, P is the pressure, and V is the system's volume.

Gibbs went one step further than Helmholtz, and defined a quantity which would also take into account the fact that when a chemical reaction takes place, heat is exchanged with the surroundings. Gibbs defined his free energy by the relation

$$G = U + PV - TS \tag{7.6}$$

or

$$G = H - TS \tag{7.7}$$

where S is the entropy of a system, H is its enthalpy, and T is its temperature.

Gibbs' reason for introducing the quantity G is as follows: The second law of thermodynamics states that in any spontaneous process, the entropy of the universe increases. Gibbs invented a simple model of the universe, consisting of the system (which might, for example, be a beaker within which a chemical reaction takes place) in contact with a large thermal reservoir at constant temperature. The thermal reservoir could, for example, be a water bath so large that whatever happens in the chemical reaction, the temperature of the bath will remain essentially unaltered. In Gibbs' simplified model, the entropy change

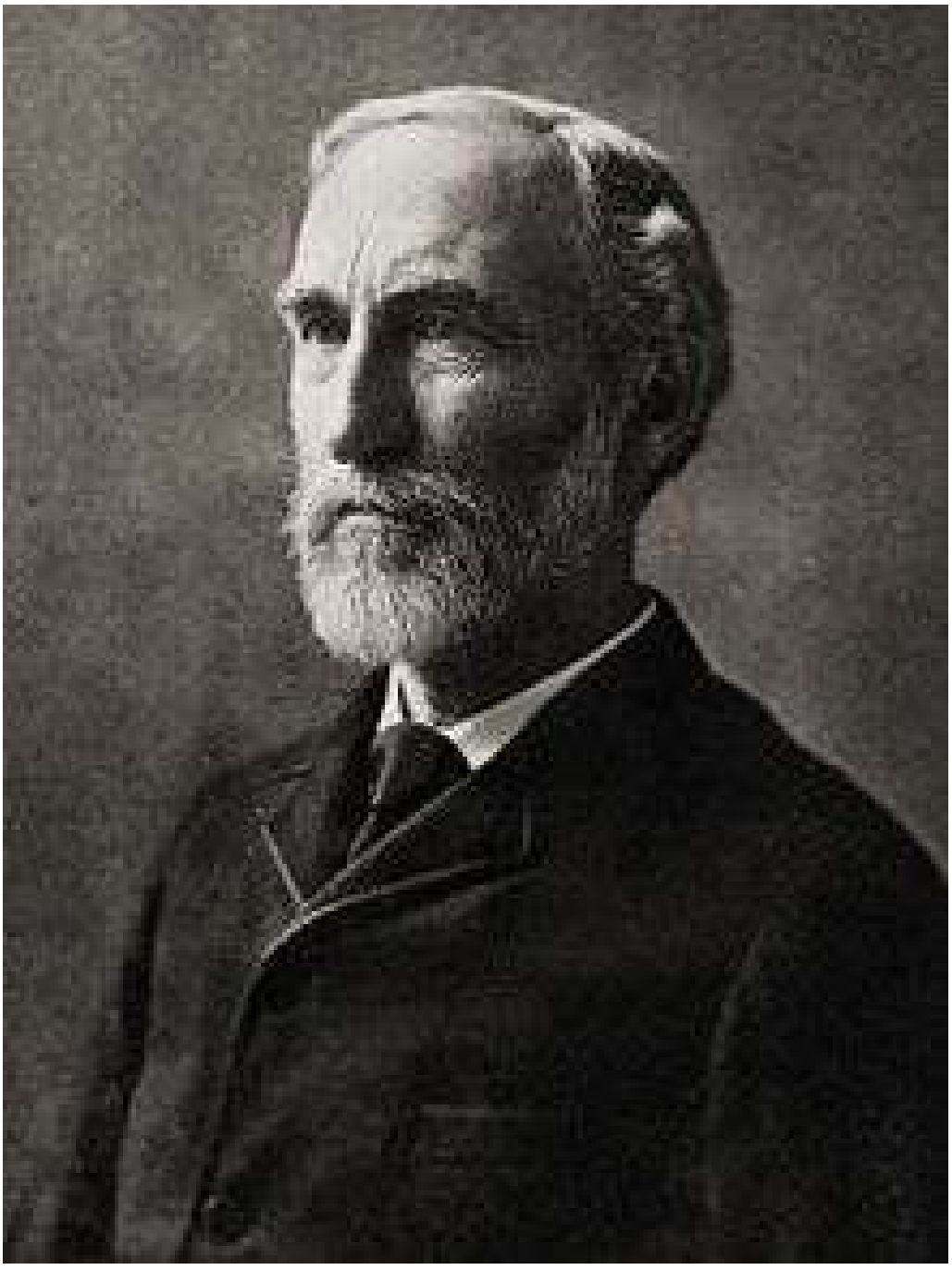

Figure 7.4: **Josiah Willard Gibbs (1839-1903). He found a way to apply thermodynamics to chemistry.**

of the universe produced by the chemical reaction can be split into two components:

$$\Delta S_{universe} = \Delta S_{system} + \Delta S_{bath} \tag{7.8}$$

Now suppose that the reaction is endothermic (i.e. it absorbs heat). Then the reaction beaker will absorb an amount of heat ΔH_{system} from the bath, and the entropy change of the bath will be

$$\Delta S_{bath} = -\frac{\Delta H_{system}}{T} \tag{7.9}$$

Combining (13.8) and (13-9) with the condition requiring the entropy of the universe to increase, Gibbs obtained the relationship

$$\Delta S_{universe} = \Delta S_{system} - \frac{\Delta H_{system}}{T} > 0 \tag{7.10}$$

The same relationship also holds for exothermic reactions, where heat is transferred in the opposite direction. Combining equations (13.38) and (13.35) yields

$$\Delta G_{system} = -T\Delta S_{universe} < 0 \tag{7.11}$$

Thus, the Gibbs free energy for a system must decrease in any spontaneous chemical reaction or process which takes place at constant temperature and pressure.

Measured values of the "Gibbs free energy of formation", ΔG_f°, are available for many molecules. To construct tables of these values, the change in Gibbs free energy is measured when the molecules are formed from their constituent elements. The most stable states of the elements at room temperature and atmospheric pressure are taken as zero points. For example, water in the gas phase has a Gibbs free energy of formation

$$\Delta G_f^\circ(H_2O) = -228.59 \ \frac{\text{kJ}}{\text{mol}} \tag{7.12}$$

This means that when the reaction

$$H_2(g) + \frac{1}{2}O_2(g) \rightarrow H_2O(g) \tag{7.13}$$

takes place under standard conditions, there is a change in Gibbs free energy of ΔG° = –228.59 kJ/mol [1]. The elements hydrogen and oxygen in their most stable states at room temperature and atmospheric pressure are taken as the zero points for Gibbs free energy of formation. Since ΔG° is negative for the reaction shown in this equation, the reaction is spontaneous. In general, the change in Gibbs free energy in a chemical reaction is given by

$$\Delta G^\circ = \sum_{products} \Delta G_f^\circ - \sum_{reactants} \Delta G_f^\circ \tag{7.14}$$

where ΔG_f° denotes the Gibbs free energy of formation.

[1] The superscript $^\circ$ means "under standard conditions", while kJ is an abbreviation for joule$\times 10^3$.

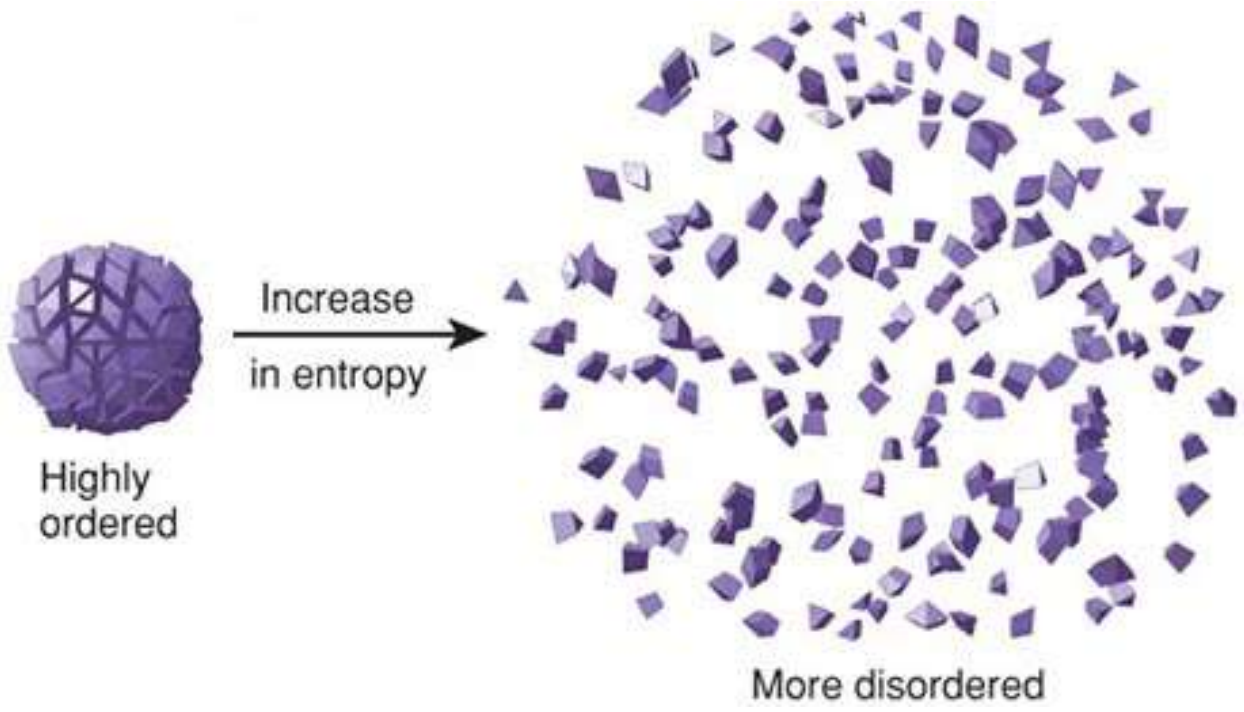

Figure 7.5: **According to the second law of thermodynamics, the entropy of the universe constantly increases. Increase of entropy corresponds to increase of disorder, and also to in- crease of statistical probability. Living organisms on the earth are able to achieve a high degree of order and highly improbable structures because the earth is not a closed system. It constantly receives free energy (i.e. energy capable of doing work) from the sun, and this free energy can be thought of as carrying thermodynamic information, or "negative entropy".**

As a second example, we can consider the reaction in which glucose is burned:

$$C_6H_{12}O_6(s) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(g) \qquad \Delta G^\circ = -2870 \ \frac{\text{kJ}}{\text{mol}} \tag{7.15}$$

The oxidation of glucose illustrates the importance of enzymes and specific coupling mechanisms in biology. A lump of glucose can sit for years on a laboratory table, fully exposed to the air. Nothing will happen. Even though the oxidation of glucose is a spontaneous process - even though the change in Gibbs free energy produced by the reaction would be negative - even though the state of the universe after the reaction would be much more probable than the initial state, the reaction does not take place, or at least we would have to wait an enormously long time to see the glucose oxidized, because the reaction pathway is blocked by potential barriers.

7.6 Svante Arrhenius

Svante Augustus Arrhenius was born in Wik Castle, Sweden in 1859, the son of Svante Gustav and Carolina Thunberg Arrhenius. He was a child prodigy, who without encouragement from his parents, taught himself to read at the age of 3. As a very young child, he also became an arithmetical prodigy by watching his father add numbers in his account books.

Arrhenius started research at the University of Uppsala, but he was dissatisfied with the instruction in physics and chemistry. In 1881 he moved to the Swedish Academy of Sciences in Stockholm. There he produced a Ph.D. dissertation which focused on conductivity of electrolytes. The dissertation was so contrary to the chemical ideas of the time that it was accepted only grudgingly by the committee judging it, and Ahrrenius was only granted a 4th class degree. Nevertheless, the 56 propositions put forward in the dissertation are universally accepted today, almost entirely without modification, and they won Ahrrenius the 1903 Nobel Prize in Chemistry.

Michael Faraday (1791-1867) had previously shown that charged particles, which he named "ions", could carry an electrical current through a solution. Ahrrenius developed Faraday's concept of ions by demonstrating that when salts are dissolved in water, ions are present even without an electrical current. He also defined acids to be substances which produce solutions in which H^+ ions predominate, while in bases, when dissolved, produce solutions in which OH^- ions predominate.

In chemical reaction theory, Ahrrenius introduced the idea of an activation energy, E_a, which can be thought of as the height of an energy barrier which must be surmounted in order for the reaction to take place. Thus most chemical reactions become more probable when the temperature T is raised, since the rapid motion of the reactants at higher temperatures can supply the energy needed to overcome the reaction barrier E_a. Ahrrenius connected the concept of activation energy with the statistical mechanics of Ludwig

Figure 7.6: **Svante Arrhenius (1859-1927) was one of the main founders of physical chemistry and a pioneer of climate science. He was related to climate activist Greta Thunberg, and Greta's father is named after him.**

Boltzmann (1844-1906) by means of his famous equation:

$$K = A\ e^{-E_a/RT}$$

In the Ahrrenius equation, K is the reaction rate, A is a constant proportional to the frequency of reactant collisions with the proper orientation, T is the absolute temperature, and R is the constant that appears in the equation of state of a perfect gas, $PV = nRT$.

7.7 The role of water in biological specificity

Below is a paper based on a lecture that I gave at a conference in Sorrento, Italy. The lecture discusses the role of water in biological specificity. In 1984 a paper based on the lecture was published in the International Journal of Quantum Chemistry. The paper has also been translated into Czech, and published in the Journal of the Czech Academy of Sciences.

To understand the role of water in biological specificity, let us imagine two opposite electrical charges in an aqueous environment. If the water were not there, the attraction between the two opposite charges would fall off as the square of the distance between them. However, there are water molecules between the two opposite charges, and to find the effective forces, we must consider the Gibbs free energy, $G = U + PV - TS$, of the total system, including the water. When two opposite electrical charges are in an aqueous

environment, the water molecules separating them become aligned so that their electric dipole moments point in the direction of the electric field. This alignment lowers the entropy of the system and raises its Gibbs free energy. Thus an effective force is produced in a direction that will lower the Gibbs free energy by reducing the volume of polarized water. This force acts strongly over a much larger distance than a simply Coulomb force. Thus the two opposite charges, which might be excess charges on the active site of an enzyme and its substrate, or an antigen and an antibody, are drawn together by the thermodynamic force that seeks to minimize the number of polarized water molecules separating them.

This thermodynamic effective force explains how the important biological processes such as auto-assembly of structures. or enzymatic activity can function so efficiently. It is because the thermodynamic forces function strongly over a much longer range than Coulomb forces, and they draw the complementary charges on the enzyme and substrate molecules, or antigen-antibody molecules, together with efficiency over much longer distances than Coulomb attraction alone could achieve.

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A Model for Biological Specificity*

JOHN AVERY

Department of Physical Chemistry, H. C. Ørsted Institute, University of Copenhagen, Copenhagen, Denmark

Abstract

The phenomenon of biological specificity is described, and a history of discoveries related to the phenomenon is presented. Aspects of biological specificity described include the mechanism of the immune system, chemotherapy, enzyme-substrate specificity, neurotransmitters, autoassembly of viruses, autoassembly of subcellular organelles, differentiation, and cellular recognition. A model for biological specificity involving both steric and electrostatic complementarity is presented and the role of structured water and hydrophobic forces is also discussed.

During the coming week, the lectures at this meeting will deal with biological topics. Most of us here are quantum chemists or physicists—That is certainly what I am myself. If we wish to apply our methods to biological problems, we are faced with a dilemma: The difficulty is that both quantum chemistry and biology are subjects which require a whole lifetime to learn thoroughly, so that it is impossible for any single person to have a deep knowledge of both fields. So what are we to do? Almost the only possibility available to us is to collaborate with a biologist or a biochemist. In such a partnership, each person has to learn enough of the other's field so that they can talk together. I hope that this lecture will serve as a contribution to the effort which we as quantum chemists must make to learn some biology. We need to make this effort in order to have biologists as friends and collaborators, and in order to appreciate the remarkable things which are happening in their field.

In this lecture, I would like to review the history of discoveries and ideas related to biological specificity. I hope in this way to convince you that the phenomenon of specificity is extremely widespread and fundamental in the operation of biological systems. I hope to show that it is involved not only in the mechanism of the immune system, but also in the mechanism of chemotherapy, in enzyme-substrate specificity, in the mechanism of neurotransmitters, in the autoassembly of viruses, in the autoassembly of subcellular organelles, in differentiation and cellular recognition, in the senses of taste and smell, and in hormone-receptor specificity. Finally, I would like to present a model of biological specificity—a model which involves both steric and electrostatic complementarity; and I will try to discuss briefly the role of structured water and hydrophobic forces.

Let us begin by looking at the history of immunology and chemotherapy. The first important discovery in this field was made by Edward Jenner in the 18th

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century. It had been known for a long time that a person who had once been infected by smallpox and who had recovered was afterwards immune to the disease. In ancient China, a powder was made from dry crusts taken from cases of smallpox, and this powder was sniffed up the nose. The result was usually a mild case of smallpox, and the innoculated person was afterwards immune. The practice of innoculation against smallpox was brought to England in 1717 by Lady Mary Montagu, the wife of the Bristish Ambassador to Turkey. This method was like Russian roulette, because it sometimes produced a fatal case of the disease. However, in 1796, Edward Jenner demonstrated that it was possible to produce immunity to smallpox by innoculation with cowpox, a much milder disease.

The discovery of a safe method of vaccination against smallpox was greeted with enormous enthusiasm everywhere in Europe. The British Parliament voted Jenner a reward of £30,000, his birthday was celebrated as a holiday in Germany, and in Russia, the first child to be vaccinated was named Vaccinov and was educated at the expense of the state.

Jenner's discovery greatly influenced Louis Pasteur. He studied Jenner's papers with extreme care and he speculated continually about how a method of safe vaccination could be found for other diseases besides smallpox. Pasteur finally was able to develop vaccines for several diseases, including anthrax and rabies, and he established general methods for preparing vaccines. We would now explain Pasteur's methods by saying that when bacteria are grown under certain abnormal conditions, a few mutant bacteria are favored by the conditions of growth. The mutants multiply, and the normal bacteria disappear. The mutant bacteria are unable to cause a serious case of the disease, but they nevertheless have antigens on their surfaces which are able to provoke a response of the immune system.

The first real understanding of the mechanism of the immune system was due to the work of Paul Ehrlich and Ilya Mechnikov, and in 1908 they shared a Nobel Prize for this work. Paul Ehrlich can be said to be the discoverer of biological specificity. As a young medical student at the University of Strasbourg, he was fortunate to work under the distinguished chemist Heinrich von Waldeyer, who took a great interest in Ehrlich. Stimulated by Waldeyer, Ehrlich began to do experiments in which he prepared thin slices of various tissues for microscopic examination by staining them with the newly discovered aniline dyes. During the last half of the 19th century, there was a great deal of interest in histological staining. It was during this period that Walther Flemming in Germany discovered chromosomes by staining them with special dyes, and Christian Gram in Denmark showed that bacteria can be classified into two types by staining methods. (We now call these two types "gram positive" and "gram negative"). During this same period, and while he was still a student, Paul Ehrlich made the important discovery that mammalian blood contains three different types of white cells which can be distinguished by staining.

Ehrlich's early work on staining made him famous, and it also gave him a set of theories which led him to his great discoveries in immunology and

chemotherapy. According to Ehrlich's ideas, the color of the aniline dyes is due to the aniline ring. However, dyes used commercially must also adhere to fabrics, and this adherence, according to Ehrlich, is due to the specific structure of the side chains. If the pattern of atoms on a side chain is complementary to the pattern of atoms on the binding site, the dye will adhere, but otherwise not. Thus there is a "lock and key" mechanism, and for this reason dyes with specific side chains stain specific types of tissue.

In one of his experiments, Paul Ehrlich injected methylene blue into the ear of a living rabbit, and found that it stained only the nerve endings of the rabbit. Since the rabbit seemed to be unharmed by the treatment, the experiment suggested to Ehrlich that it might be possible to find antibacterial substances which could be safely injected into the bloodstream of a patient suffering from an infectious disease. Ehrlich hoped to find substances which would adhere selectively to the bacteria, while leaving the tissues of the patient untouched.

With the help of a large laboratory especially constructed for him in Frankfurt, the center of the German dye industry, Ehrlich began to screen thousands of modified dyes and other compounds. In this way he discovered trypan red, a chemical treatment for sleeping sickness, and arsphenamine, a drug which would cure syphilis. Ehrlich thus became the father of modern chemotherapy. His success pointed the way to Gerhard Domagk, who discovered the sulphonamide drugs in the 1930s, and to Fleming, Waksman, Dubos and others, who discovered the antibiotics.

Ehrlich believed that in the operation of the immune system, the body produces molecules which have a pattern of atoms complementary to patterns (antigens) on invading bacteria, and that these molecules (antibodies) in the blood stream kill the bacteria by adhering to them. Meanwhile, the Russian naturalist Ilya Mechnikov discovered another mechanism by which the immune system operates. While on vacation in Sicily, Mechnikov was studying the digestive process in starfish larvae. In order to do this, he introduced some particles of carmine into the larvae. The starfish larvae were completely transparent, and thus Mechnikov could look through his microscope and see what happened to the particles. He saw that they were enveloped and apparently digested by wandering amoebalike cells inside the starfish larvae. As he watched this process, it suddenly occurred to Mechnikov that our white cells might similarly envelop and digest bacteria, thus protecting us from infection. Describing this discovery, Mechnikov wrote in his diary: "I suddenly became a pathologist! Feeling that there was in this idea something of surpassing interest, I became so excited that I began striding up and down the room, and even went to the seashore to collect my thoughts."

Mechnikov later named the white cells "phagocytes" (which means "eating cells"). He was able to show experimentally that phagocytosis (i.e., the envelopment and digestion of bacteria by phagocytes) is an important mechanism in immunity. For a number of years, there were bitter arguments between those who thought that the immune system operates through phagocytosis, and those who thought that it operates through antibodies. Finally it was found that both mechanisms play a role. In phagocytosis, the bacterium will not be ingested by

the phagocyte unless it is first studded with antibodies. Thus both Mechnikov and Ehrlich were proved to be right.

Early in the 20th century, important work in immunology was done by Karl Landsteiner, who won the 1930 Nobel Prize in medicine and physiology for his discovery of the human blood groups. His book, entitled *The Specificity of Serological Reactions,* is listed in the references [1]. In 1936, Landsteiner asked Linus Pauling (who was then visiting the Rockefeller Institute for Medical Research), to try to develop a theory which would account for antibody-antigen specificity in the operation of the immune system [2]. The result was a theory by Pauling, in which some features were correct, but others badly wrong. Pauling decided that "... The specific combining region of an antibody molecule is complementary in structure to a portion of the surface of the antigen, with the antigen-antibody bond resulting from the cooperation of weak forces (electronic van der Waals forces, electrostatic interaction of charged groups, and hydrogen bonding) between the complementary structures, over an area sufficiently large that the total binding energy can resist the disrupting influence of thermal agitation." This much of Pauling's 1940 theory is today considered to be correct. However, Pauling also made the hypothesis—and this is where he went wrong—that in the immune system, the antigen serves as a template for the construction of the antibody (in much the same way that a DNA strand serves as a template for the construction of the complementary strand). Once the lymphocytes have "learned" how to produce antibodies fitting a particular antigen, Pauling believed, they continue to produce them, and thus we become immune [3].

Pauling's reason for believing in a template theory of antibody formation was the enormous range of specificities which can be matched. The mammalian immune system can produce antibodies of roughly 10^7 different specificities. It seemed impossible to Pauling that so many different specificities could be genetically coded. However, subsequent research [4–6] has shown that the capability for producing this immense variety of antibodies is, in fact, genetically programmed. Each lymphocyte produces its own specific antibody molecule, and when a lymphocyte divides, the daughter cells continue to produce exactly the same antibody. Animals of a particular species, when challenged with a particular antigen, may be unable to produce an antibody against it, while animals of a slightly different genetic strain, when challenged with the same antigen, can readily produce the appropriate antibody.

Thus, Pauling's template theory of immunity had to be abandoned. It was replaced by the clonal theory of Niels Kai Jerne and Sir Frank MacFarlane Burnet. According to the clonal theory of immunity, which is the currently accepted theory, a few lymphocytes corresponding to each of the 10^7 different specificities are present in a nonimmune individual. When the individual becomes ill with an infection, antigens on the surfaces of the invading microorganisms bind to antibody molecules on the surfaces of just those lymphocytes which have the right specificity. This stimulates the selected lymphocytes to divide rapidly, and after a period of time, a population of lymphocytes capable of producing the correct antibody builds up. When this happens, the infected individual

recovers. Even after recovery, a substantial population of that strain of lymphocyte remains, and if the individual is again invaded by the same type of microorganism, this population of lymphocytes can immediately produce the appropriate antibody. an individual with this capability is immune.

The clonal theory of immunity has an interesting consequence: Because of the fact that when a lymphocyte divides, the daughter cells produce exactly the same antibody as the parent, it follows that if one could culture lymphocytes, one could produce pure antibodies *in vitro*. However, if one tries to culture these cells in a direct way, they die after a few generations. In 1975, Georges Köhler and Cesar Milstein succeeded in culturing lymphocytes by fusing them with myeloma cancer cells. The resulting hybrid cell lines were immortal, and cultures from single cells could be grown indefinitely, producing pure "monoclonal" antibodies [6–15].

The monoclonal antibody technique of Köhler and Milstein allows one to separate mixtures of unknown composition into their components. This is done in the following way: A mouse is immunized with the mixture, and spleen cells from the mouse are fused with myeloma cells. The hybrid cells are spread out into several hundred small culture dishes, one cell to each dish. After a clone has grown from the single cell in each dish, the supernatants are reacted one at a time with the mixture. Each component of the mixture makes an insoluble product with a different supernatant, and thus the mixture is separated into its components.

The monoclonal antibody technique is an extremely powerful tool, which can be used in the purification of proteins, the characterization of viruses, the treatment of cancer, in genetic studies, and in many other applications.

Until now, we have been considering only immunology and chemotherapy as examples of biological specificity. However, specificity is a much more general and fundamental phenomenon in biology. For example, one can see the phenomenon in operation in the autoassembly of viruses and subcellular organelles. Fraenkel-Conrat [16] has shown that by changing the pH, it is possible to take a virus to pieces. When the original pH is restored, the pieces spontaneously reassemble themselves into a virus capable of producing an infection. A similar spontaneous assembly must also occur whenever a virus reproduces itself. After the constituent parts have been manufactured by the ribosomes of the host cell, they must come together spontaneously. This process is analogous to crystallization, but more complicated, since the virus contains molecules of several different kinds. How can the pieces "know" enough to fit themselves together? The answer must be that regions on each constituent molecule of a virus are complementary to regions on the neighboring molecule of the finished structure, so that they bind selectively to the right place, and perhaps are even attracted to the right place. The same kind of spontaneous assembly, analogous to crystallization, must occur in the autoassembly of subcellular organelles, such as chloroplasts and mitochondria.

Specificity is also important in the operation of the central nervous system. A number of different substances are released at synapses (for example, acetylcho-

line, noradrenalin, serotonin, and dopamine). These neurotransmitter substances can stimulate or inhibit the firing of the next neuron, each substance being specific to a particular type of receptor on the neighboring neuron [17–20].

Cell surface antigens are involved in differentiation during the development of an embryo. For example, the H-Y antigen (a pattern of atoms which is present on the plasma membrane of all male mammalian cells) is known to be a differentiation antigen. The H-Y antigen [21–30] has been shown to be present on the cell surfaces of male mammalian embryos at the eight-cell stage, and it has been shown to be involved in the development of the embryo into a male, long before testosterone is present in the embryo. If the H-Y antigen is absent, the embryo develops into a female. Interestingly, the H-Y antigen seems to play a similar role in birds, reptiles, and amphibians; but in birds, it occurs on the cells of the female, and in amphibians, sometimes on the cells of one sex, and sometimes the other. This irregularity is only superficial, however, since the H-Y antigen is invariably linked to the development of the heterogametic sex. In the case of mammals, the male is heterogametic; in the case of birds, the female is heterogametic; and in the case of amphibians, the heterogametic sex is variable, depending on the species.

Other areas of biology where specificity plays an important role include the senses of taste and smell [31, 32], enzyme-substrate interactions [33–35] and hormone-receptor interactions.

I would like to end this lecture by proposing a model for biological specificity. The model consists of three assertions: (1) The complementarity involved in biological specificity is, in general, both steric and electrostatic. (2) There is a matching of nonpolar regions. (3) The total system, including water molecules, tends to move in such a way that its Gibbs free energy, $G = E + PV - TS$, decreases.

The last point in the model has been called the "thermodynamic hypothesis" by Anfinsen [36], and he has shown that it holds in the folding of proteins. ("Hypothesis" is almost too modest a name for the rule that the Gibbs free energy of a system tends to decrease, since this rule is one of the main guiding principles of theoretical chemistry.) One can even define a "thermodynamic force," as has been done by Buckingham and McLachlan [37–40]. If the Gibbs free energy G is a function of N coordinates, $x_1, x_2, \ldots, x_N$ (which might represent nuclear coordinates), then the thermodynamic force corresponding to one of the coordinates is given by $\partial G/\partial x_i$. The direction of this force gives the direction in which the system tends to move, according to the thermodynamic hypothesis. However, one should remember that this is not the same kind of force which enters Newton's equations.

The first point in the model does not mention dispersion forces. This is not because dispersion forces are always negligibly small, but because it is hard to visualize complementarity with respect to dispersion forces. In cases where dispersion forces are important, it is steric complementarity which allows the two specific combining regions to come close enough to each other so that the dispersion forces are effective. Hydrogen bonds also go unmentioned in the first point of the model, but this is because they are included under the heading of electrostatic complementarity.

As Professor Tomasi has emphasized in his lecture, when two molecules approach each other but are not yet in contact, the classical electrostatic interaction between them is often the dominant term in the interaction energy [41]. Alberte and Bernard Pullman have also emphasized the importance of electrostatic interactions [42–45]. Thus, when we visualize the interaction between, for example, an enzyme and its substrate as they approach each other, we should visualize the interaction as being initially primarily electrostatic. Only after the approach has become very close (~1–2 Å), will other types of forces become important.

We must now ask what role the solvent water molecules will play. The large variety of ways in which a water molecule can form hydrogen bonds with its neighbors contributes to the entropy of water. When this freedom to form hydrogen bonds in many ways is restricted, the entropy is decreased. If we introduce a nonpolar molecule into water, the water molecules around it become more highly ordered and "icelike," the variety of ways in which they form hydrogen bonds is limited, and thus the entropy is decreased. This is the reason for the well-known insolubility of nonpolar molecules in water [49, 50]. The entropy term in the Gibbs free energy,

$$G = E + PV - TS, \tag{1}$$

favors configurations in which the contact of water with nonpolar molecules or groups is minimized. This hydrophobic effect has the consequence that in biological specificity, nonpolar regions of combining sites tend to come together in order to escape contact with water (point 2 of the model).

The entropy of water is also reduced when the water molecules are aligned by an electric field. Water has a high dielectric constant, which is due to the dipole moment formed by the positively charged hydrogens and the negatively charged oxygen lone pairs [46–62]. When two charges interact with each other in an aqueous medium, the intervening water molecules align themselves with their dipole moments pointing in such a way that the interaction energy of the two charges is reduced. Thus, at first sight, it would seem that the effect of the polarized water between two charges would be to very much reduce their attraction for each other. We should remember, however, that the Gibbs free energy of the system also contains an entropy term, and this term has the opposite effect. When water molecules are aligned in the electric field, their entropy is lowered. If the system tends in its motion towards a state with the lowest possible Gibbs free energy, it will prefer a state where the number of oriented water molecules is reduced. Thus the entropy term in the Gibbs free energy tends to make the "thermodynamic force" between two charges stronger, canceling at least part of the effect of the dielectric constant.

One can easily calculate the entropy of a system of N dipoles in an external field if one makes the simplifying assumption that the dipoles have only two quantum states, one parallel to the applied field, and the other antiparallel, differing by the energy $\Delta E = \mu F$. (F is the effective electric field acting on the dipole, i.e., it is due partly to the external field and partly to the fields of the

other dipoles in the system.) then using the relation

$$S=\frac{E}{T}+k\ln Q \tag{2}$$

(where k is Boltzmann's constant, T is the temperature, E is the energy of the system, and Q is its partition function) we obtain

$$S=Nk\left(\frac{xe^{-x}}{1+e^{-x}}+\ln\left(1+e^{-x}\right)\right),$$
$$x\equiv\frac{\Delta E}{kT} \tag{3}$$

The behavior of this entropy as a function of x is as shown in Figure 1.

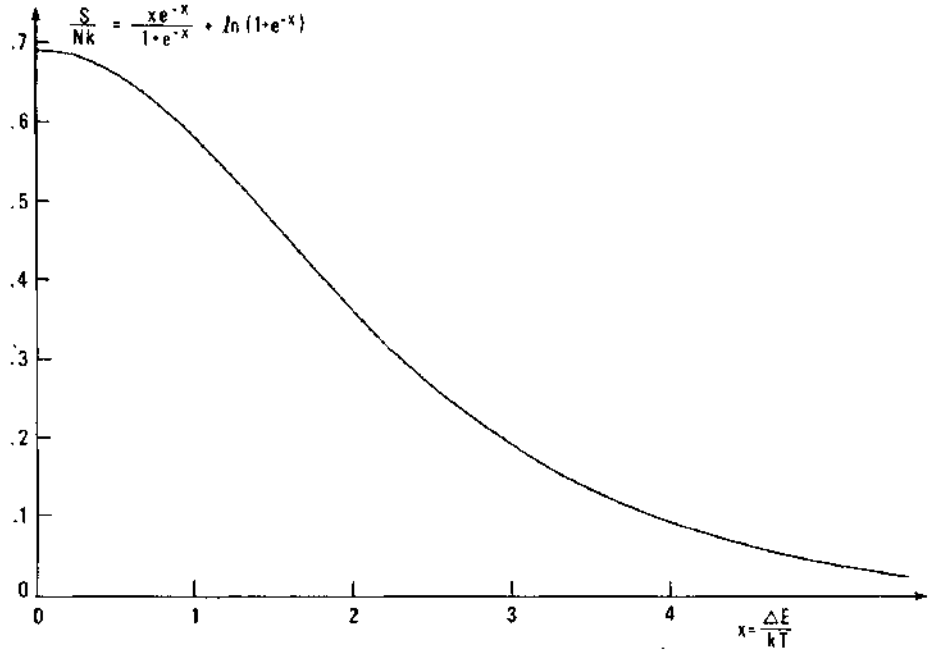

Figure 1. The entropy of a system of electric dipoles as a function of the electric field strength, under the simplifying assumption that the dipoles have only two possible quantum states, one parallel and the other antiparallel to the field.

The simple example discussed above cannot give us more than an extremely rough and qualitative picture of how the entropy of water behaves as a function of electric field strength. Some further insight can be obtained by considering the entropy change which takes place when ice *Ic* is placed in a strong electric field. Ice *Ic* (cubic ice) is a form of ice in which the oxygen atoms are arranged in a structure isomorphous with the arrangement of carbon in diamond [48].

Each oxygen atom in ice *Ic* is tetrahedrally hydrogen bonded to four other oxygen atoms. The distance between neighboring oxygen atoms is 2.76 Å.

In 1935, Linus Pauling [63, 64] published a paper on the low-temperature entropy of ice in which he argued that the water molecule is essentially intact in ice. In the gas phase, the H—O bond length in water is 0.95 Å. Pauling argued that "the magnitudes of changes in properties from steam to ice are not sufficiently great to permit us to assume that this distance is increased to 1.38 Å." Therefore, Pauling argued, in ice, a hydrogen atom between two oxygens is not placed midway between them, but is nearer to one than to the other. Pauling's hypothesis that the water molecule in ice is essentially intact was later confirmed by neutron diffraction experiments.

In his 1935 paper, Pauling showed that if the water molecules in ice are assumed to be essentially intact, the hydrogen-bonding system of the crystal can be formed in $(\frac{3}{2})^N$ different ways, where N is the number of water molecules in the crystal. He showed that this large variety of possible conformations of the crystal, none of which differs appreciably in energy from the others, gives rise to a residual low-temperature entropy of

$$\Delta S = Nk \ln (\tfrac{3}{2}) = 0.805 Nk, \tag{4a}$$

where k is Boltzmann's constant. This calculated residual low-temperature entropy is close to the measured value of $0.87Nk$, an agreement which gives strong support to Pauling's theory.

Now let us consider what happens when ice is placed in an electric field which is strong enough to produce total orientation of the dipoles, but which nevertheless leaves the water molecules essentially intact. Can the water molecules reorient themselves in such a way that all the molecules have large components of their dipole moments pointing in the direction of the field, while still maintaining the hydrogen bonding system? From Figure 2, we can see that this is possible, but that there is only one possible configuration in which the dipoles are correctly oriented. In other words, in an electric field which is strong enough to produce total orientation of the water molecules, the residual low temperature entropy drops to zero, and the entropy change produced by applying the field is given by Eq. (4a). for smaller field strengths, the entropy would be difficult to calculate, but presumably it would fall off as a function of field strength in the manner of the entropy of the system of dipoles shown in Figure 1.

The two simple systems discussed above can give us a certain amount of qualitative insight into the behavior of the entropy of water as a function of applied electric field. However, it would be very desirable to have experimental determinations of the entropy and energy of water in strong electric fields. This information would be needed if one were to attempt to calculate the thermodynamic force between two charged particles in an aqueous medium.

If electrostatic forces are important in biological specificity, one might ask how far such forces extend. It might be possible to answer this question experimentally, starting with a knowledge of the diffusion constants of the molecules

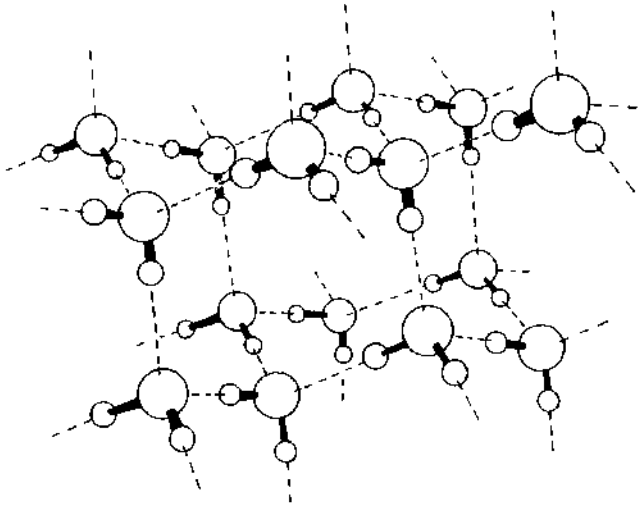

Figure 2. The hydrogen bonding system in ice *Ic*. When a strong electric field is applied, the molecules can orient themselves in such a way that each molecule has a large component of its dipole moment pointing in the direction of the field, while still maintaining the hydrogen bonding system. However, there is only one conformation in which this is possible (that shown in the figure); and thus, application of a strong electric field reduces the low-temperature residual entropy to zero.

involved in (for example) antigen-antibody reactions or enzyme-substrate reactions. It might then be possible to calculate the time which would be needed for binding under the assumption that the components had to reach the correct position and orientation by entirely random Brownian motion. The rate of binding could afterwards be calculated under the assumption that electrostatic forces reach out a certain distance into the solution, so that if the components diffuse to within a certain distance of one another, and to within a certain difference from the correct orientation, they will be trapped. In other words, the binding rate would be calculated under the assumption that if the reactants diffused to within a certain critical distance and critical error of orientation from the correct position, they would have very little probability of escaping, and would almost inevitably be drawn in and correctly oriented by electrostatic forces. These two binding rates could be compared with observed rates, and from this comparison, the degree to which electrostatic forces reach out into the solution and draw the components into place could be estimated.

Experiments and calculations might also be aimed at examination of the binding sites responsible for specificity, to determine whether or not electrostatic complementarity is involved. The crystallographic structures of a number of enzymes are known. For example, the structure of lysozyme has been determined by D. C. Phillips and co-workers [65]. As Professor Ricard has pointed out [35], the binding site of an enzyme is more closely complementary to an inhibitor than it is to the equilibrium conformation of its substrate. As the substrate of an

enzyme-mediated reaction approaches the binding site, forces exerted by the site distort the substrate in the direction of the transition state, thus reducing the activation energy for the reaction. Notice that this picture implies the existence of forces which extend some distance out from the site. In cases where two reactants are joined together by an enzyme, such forces may help to guide the reactants together in the proper orientation, a mechanism which Koshland has called "orbital steering" [34].

From x-ray crystallographic data, it is possible to construct the electrostatic potential [66, 67]. To do this, one represents the charge density $\rho(x)$ by a Fourier series of the form:

$$\rho(\mathbf{x}) = \sum_{\mathbf{K}} (\rho)_{\mathbf{K}} \, e^{i\mathbf{K}\cdot\mathbf{x}}, \tag{4}$$

where the vectors $\mathbf{K}$ are reciprocal lattice vectors. Essentially, the Fourier coefficients $(\rho)_{\mathbf{K}}$ are what is measured in an x-ray diffraction experiment. Since the charge density and the electrostatic potential $\phi(\mathbf{x})$ are related through Poisson's equation:

$$\nabla^2\phi = -4\pi\rho. \tag{5}$$

It follows that if $\phi(\mathbf{x})$ is represented by the Fourier series

$$\phi(\mathbf{x}) = \sum_{\mathbf{K}} (\phi)_{\mathbf{K}} \, e^{i\mathbf{K}\cdot\mathbf{x}}, \tag{6}$$

the Fourier coefficients are related by

$$(\phi)_{\mathbf{K}} = \frac{4\pi}{K^2}(\rho)_{\mathbf{K}}. \tag{7}$$

Thus crystallographic measurements of Fourier coefficients of the charge density can be used to construct electrostatic fields. This method could be used to examine the active sites of enzymes to determine the electrostatic potentials near to the sites. Alternatively, it might be possible to calculate the charge distributions and potentials quantum mechanically, using methods such as those described by Professor McWeeny in his lecture [68–74].

I hope that future work in this direction will throw some light onto the phenomenon of biological specificity, one of the most widespread and fundamental phenomena in biology. In the meantime, I would tentatively put forward the view that in biological specificity, the molecules involved do not have to cover the entire distance to their binding sites by random diffusion. Perhaps during the last steps of the journey, they are guided into place by relatively long-range thermodynamic forces involving the entropy and energy of the intervening water molecules.

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Chapter 8

SOME RECENT DEVELOPMENTS

8.1 Gene splicing

In 1970, Hamilton Smith of Johns Hopkins University observed that when the bacterium *Haemophilus influenzae* is attacked by a bacteriophage (a virus parasitic on bacteria), it can defend itself by breaking down the DNA of the phage. Following up this observation, he introduced DNA from the bacterium *E. coli* into *H. influenzae*. Again the foreign DNA was broken down.

Further investigation revealed that *H. influenzae* produced an enzyme, later named *Hin* dII, which cut a DNA strand only when it recognized a specific sequence of bases: The DNA was cut only if one strand contained the sequence GTPyPuAC, where Py stands for C or T, while Pu stands for A or G. The other strand, of course, contained the complementary sequence, CAPuPyTG. The enzyme *Hin* dII cut both strands in the middle of the six-base sequence.

Smith had, in fact, discovered the first of a class of bacterial enzymes which came to be called "restriction enzymes" or "restriction nucleases". Almost a hundred other restriction enzymes were subsequently discovered; and each was found to cut DNA at a specific base sequence. Smith's colleague, Daniel Nathans, used the restriction enzymes *Hin* dII and *Hin* dIII to produce the first "restriction map" of the DNA in a virus.

In 1971 and 1972, Paul Berg, and his co-workers Peter Lobban, Dale Kaiser and David Jackson at Stanford University, developed methods for adding cohesive ends to DNA fragments. Berg and his group used the calf thymus enzyme, terminal transferase, to add short, single-stranded polynucleotide segments to DNA fragments. For example, if they added the single-stranded segment AAAA to one fragment, and TTTT to another, then the two ends joined spontaneously when the fragments were incubated together. In this way Paul Berg and his group made the first recombinant DNA molecules.

The restriction enzyme *Eco* RI, isolated from the bacterium *E. coli*, was found to recognize the pattern, GAATTC, in one strand of a DNA molecule, and the complementary pattern, CTTAAG, in the other strand. Instead of cutting both strands in the middle of the six-base sequence, *Eco* RI was observed to cut both strands between G and A. Thus,

each side of the cut was left with a "sticky end" - a four-base single-stranded segment, attached to the remainder of the double-stranded DNA molecule.

In 1972, Janet Mertz and Ron Davis, working at Stanford University, demonstrated that DNA strands cut with *Eco* RI could be rejoined by means of another enzyme - a DNA ligase. More importantly, when DNA strands from two different sources were cut with *Eco* RI, the sticky end of one fragment could form a spontaneous temporary bond with the sticky end of the other fragment. The bond could be made permanent by the addition of DNA ligase, even when the fragments came from different sources. Thus, DNA fragments from different organisms could be joined together.

Bacteria belong to a class of organisms (prokaryotes) whose cells do not have a nucleus. Instead, the DNA of the bacterial chromosome is arranged in a large loop. In the early 1950's, Joshua Lederberg had discovered that bacteria can exchange genetic information. He found that a frequently-exchanged gene, the F-factor (which conferred fertility), was not linked to other bacterial genes; and he deduced that the DNA of the F-factor was not physically a part of the main bacterial chromosome. In 1952, Lederberg coined the word "plasmid" to denote any extrachromosomal genetic system.

In 1959, it was discovered in Japan that genes for resistance to antibiotics can be exchanged between bacteria; and the name "R-factors" was given to these genes. Like the F-factors, the R-factors did not seem to be part of the main loop of bacterial DNA.

Because of the medical implications of this discovery, much attention was focused on the R-factors. It was found that they were plasmids, small loops of DNA existing inside the bacterial cell, but not attached to the bacterial chromosome. Further study showed that, in general, between one percent and three percent of bacterial genetic information is carried by plasmids, which can be exchanged freely even between different species of bacteria.

In the words of the microbiologist, Richard Novick, "Appreciation of the role of plasmids has produced a rather dramatic shift in biologists' thinking about genetics. The traditional view was that the genetic makeup of a species was about the same from one cell to another, and was constant over long periods of time. Now a significant proportion of genetic traits are known to be variable (present in some individual cells or strains, absent in others), labile (subject to frequent loss or gain) and mobile - all because those traits are associated with plasmids or other atypical genetic systems."

In 1973, Herbert Boyer, Stanley Cohen and their co-workers at Stanford University and the University of California carried out experiments in which they inserted foreign DNA segments, cut with *Eco* RI, into plasmids (also cut with *Eco* RI). They then resealed the plasmid loops with DNA ligase. Finally, bacteria were infected with the gene-spliced plasmids. The result was a new strain of bacteria, capable of producing an additional protein coded by the foreign DNA segment which had been spliced into the plasmids.

Cohen and Boyer used plasmids containing a gene for resistance to an antibiotic, so that a few gene-spliced bacteria could be selected from a large population by treating the culture with the antibiotic. The selected bacteria, containing both the antibiotic-resistance marker and the foreign DNA, could then be cloned on a large scale; and in this way a foreign gene could be "cloned". The gene-spliced bacteria were chimeras, containing genes from two

different species.

The new recombinant DNA techniques of Berg, Cohen and Boyer had revolutionary implications: It became possible to produce many copies of a given DNA segment, so that its base sequence could be determined. With the help of direct DNA-sequencing methods developed by Frederick Sanger and Walter Gilbert, the new cloning techniques could be used for mapping and sequencing genes.

Since new bacterial strains could be created, containing genes from other species, it became possible to produce any protein by cloning the corresponding gene. Proteins of medical importance could be produced on a large scale. Thus, the way was open for the production of human insulin, interferon, serum albumin, clotting factors, vaccines, and protein hormones such as ACTH, human growth factor and leuteinizing hormone.

It also became possible to produce enzymes of industrial and agricultural importance by cloning gene-spliced bacteria. Since enzymes catalyze reactions involving smaller molecules, the production of these substrate molecules through gene-splicing also became possible.

It was soon discovered that the possibility of producing new, transgenic organisms was not limited to bacteria. Gene-splicing was also carried out on higher plants and animals as well as on fungi. It was found that the bacterium *Agrobacterium tumefaciens* contains a tumor-inducing (Ti) plasmid capable of entering plant cells and producing a crown gall. Genes spliced into the Ti plasmid frequently became incorporated in the plant chromosome, and afterwards were inherited in a stable, Mendelian fashion.

Transgenic animals were produced by introducing foreign DNA into embryo-derived stem cells (ES cells). The gene-spliced ES cells were then selected, cultured and introduced into a blastocyst, which afterwards was implanted in a foster-mother. The resulting chimeric animals were bred, and stable transgenic lines selected.

Thus, for the first time, humans had achieved direct control over the process of evolution. Selective breeding to produce new plant and animal varieties was not new - it was one of the oldest techniques of civilization. However, the degree and speed of intervention which recombinant DNA made possible was entirely new. In the 1970's it became possible to mix the genetic repetoires of different species: The genes of mice and men could be spliced together into new, man-made forms of life!

The Asilomar Conference

In the summer of 1971, Janet Mertz, who was then a student in Paul Berg's laboratory, gave a talk at Cold Spring Harbor. She discussed some proposed experiments applying recombinant techniques to the DNA of the tumor-inducing virus SV40.

This talk worried the cell biologist, Richard Pollack. He was working with SV40 and was already concerned about possible safety hazards in connection with the virus. Pollack telephoned to Berg, and asked whether it might not be dangerous to clone a gene capable of producing human cancer. As a result of this call, Berg decided not to clone genes from tumor-inducing viruses.

Additional concern over the safety of recombinant DNA experiments was expressed at

the 1973 Gordon Conference on Nucleic Acids. The scientists attending the conference voted to send a letter to the President of the U.S. National Academy of Sciences:

"...We presently have the technical ability", the letter stated, "to join together, covalently, DNA molecules from diverse sources... This technique could be used, for example, to combine DNA from animal viruses with bacterial DNA... In this way, new kinds of hybrid plasmids or viruses, with biological activity of unpredictable nature, may eventually be created. These experiments offer exciting and interesting potential, both for advancing knowledge of fundamental biological processes, and for alleviation of human health problems."

"Certain such hybrid molecules may prove hazardous to laboratory workers and to the public. Although no hazard has yet been established, prudence suggests that the potential hazard be seriously considered."

"A majority of those attending the Conference voted to communicate their concern in this matter to you, and to the President of the Institute of Medicine... The conferees suggested that the Academies establish a study committee to consider this problem, and to recommend specific actions and guidelines."

As a result of this letter, the National Academy of Sciences set up a Committee on Recombinant DNA, chaired by Paul Berg. The Committee's report, published in July, 1974, contained the following passage:

"...There is serious concern that some of these artificial recombinant DNA molecules could prove biologically hazardous. One potential hazard in current experiments derives from the need to use a bacterium like *E. coli* to clone the recombinant DNA molecules and to amplify their number. Strains of *E. coli* commonly reside in the human intestinal tract, and they are capable of exchanging genetic information with other types of bacteria, some of which are pathogenic to man. Thus, new DNA elements introduced into *E. coli* might possibly become widely disseminated among human, bacterial, plant, or animal populations, with unpredictable effects."

The Committee on Recombinant DNA recommended that scientists throughout the world should join in a voluntary postponement of two types of experiments: Type 1, introduction of antibiotic resistance factors into bacteria not presently carrying the R-factors; and Type 2, cloning of cancer-producing plasmids or viruses.

The Committee recommended caution in experiments linking DNA from animal cells to bacterial DNA, since animal-derived DNA can carry cancer-inducing base sequences. Finally, the Committee recommended that the National Institutes of Health establish a permanent advisory group to supervise experiments with recombinant DNA, and that an international meeting be held to discuss the biohazards of the new techniques.

In February, 1975, more than 100 leading molecular biologists from many parts of the world met at the Asilomar Conference Center near Monterey, California, to discuss safety guidelines for recombinant DNA research. There was an almost unanimous consensus at the meeting that, until more was known about the dangers, experiments involving cloning of DNA should make use of organisms and vectors incapable of living outside a laboratory environment.

The Asilomar Conference also recommended that a number of experiments be deferred.

These included cloning of recombinant DNA derived from highly pathogenic organisms, or containing toxin genes, as well as large-scale experiments using recombinant DNA able to make products potentially harmful to man, animals or plants.

The Asilomar recommendations were communicated to a special committee appointed by the U.S. National Institutes of Health; and the committee drew up a set of guidelines for recombinant DNA research. The NIH Guidelines went into effect in 1976; and they remained in force until 1979. They were stricter than the Asilomar recommendations regarding cloning of DNA from cancer-producing viruses; and this was effectively forbidden by the NIH until 1979. (Of course, the NIH Guidelines were effective only for research conducted within the United States and funded by the U.S. government.)

In 1976, the first commercial genetic engineering company (Genentech) was founded. In 1980, the initial public offering of Genentech stock set a Wall Street record for the fastest increase of price per share. In 1981, another genetic engineering company (Cetus) set a Wall Street record for the largest amount of money raised in an initial public offering (125 million U.S. dollars). During the same years, Japan's Ministry of International Trade and Technology declared 1981 to be "The Year of Biotechnology"; and England, France and Germany all targeted biotechnology as an area for special development.

A number of genetic-engineering products reached the market in the early 1980's. These included rennin, animal growth hormones, foot and mouth vaccines, hog diarrhea vaccine, amino acids, antibiotics, anabolic steroids, pesticides, pesticide-resistant plants, cloned livestock, improved yeasts, cellulose-digesting bacteria, and a nitrogen-fixation enzyme.

Recently the United States and Japan have initiated large-scale programs whose aim is to map the entire human genome; and the European Economic Community is considering a similar program. The human genome project is expected to make possible prenatal diagnosis of many inherited diseases. For example, the gene for cystic fibrosis has been found; and DNA technology makes it possible to detect the disease prenatally.

The possibility of extensive genetic screening raises ethical problems which require both knowledge and thought on the part of the public. An expectant mother, in an early stage of pregnancy, often has an abortion if the foetus is found to carry a serious genetic defect. But with more knowledge, many more defects will be found. Where should the line be drawn between a serious defect and a minor one?

The cloning of genes for lethal toxins also needs serious thought and public discussion. From 1976 to 1982, this activity was prohibited in the United States under the NIH Guidelines. However, in April, 1982, the restriction was lifted, and by 1983, the toxins being cloned included several aflatoxins, lecithinase, cytochalasins, ochratoxins, sporidesmin, T-2 toxin, ricin and tremogen. Although international conventions exist under which chemical and biological weapons are prohibited, there is a danger that nations will be driven to produce and stockpile such weapons because of fear of what other nations might do.

Finally, the release of new, transgenic species into the environment requires thought and caution. Much benefit can come, for example, from the use of gene-spliced bacteria for nitrogen fixation or for cleaning up oil spills. However, once a gene-spliced microorganism has been released, it is virtually impossible to eradicate it; and thus the change produced by the release of a new organism is permanent. Permanent changes in the environment

should not be made on the basis of short-term commercial considerations, nor indeed on the basis of short-term considerations of any kind; nor should such decisions be made unilaterally by single nations, since new organisms can easily cross political boundaries.

The rapid development of biotechnology has given humans enormous power over the fundamental mechanisms of life and evolution. But is society mature enough to use this power wisely and compassionately?

The Polymerase Chain Reaction

One day in the early 1980's, an American molecular biologist, Kary Mullis, was driving to his mountain cabin with his girl friend. The journey was a long one, and to pass the time, Kary Mullis turned over and over in his mind a problem which had been bothering him: He worked for a California biotechnology firm, and like many other molecular biologists he had been struggling to analyze very small quantities of DNA. Mullis realized that it would be desirable have a highly sensitive way of replicating a given DNA segment - a method much more sensitive than cloning. As he drove through the California mountains, he considered many ways of doing this, rejecting one method after the other as impracticable. Finally a solution came to him; and it seemed so simple that he could hardly believe that he was the first to think of it. He was so excited that he immediately pulled over to the side of the road and woke his sleeping girlfriend to tell her about his idea. Although his girlfriend was not entirely enthusiastic about being wakened from a comfortable sleep to be presented with a lecture on biochemistry, Kary Mullis had in fact invented a technique which was destined to revolutionize DNA technology: the Polymerase Chain Reaction (PCR)[1].

The technique was as follows: Begin with a small sample of the genomic DNA to be analyzed. (The sample may be extremely small - only a few molecules.) Heat the sample to 95 °C to separate the double-stranded DNA molecule into single strands. Suppose that on the long DNA molecule there is a target segment which one wishes to amplify. If the target segment begins with a known sequence of bases on one strand, and ends with a known sequence on the complementary strand, then synthetic "primer" oligonucleotides[2] with these known beginning ending sequences are added in excess. The temperature is then lowered to 50-60 °C, and at the lowered temperature, the "start" primer attaches itself to one DNA strand at the beginning of the target segment, while the "stop" primer becomes attached to the complementary strand at the other end of the target segment. Polymerase (an enzyme which aids the formation of double-stranded DNA) is then added, together with a supply of nucleotides. On each of the original pieces of single-stranded DNA, a new complementary strand is generated with the help of the polymerase. Then the temperature is again raised to 95 °C, so that the double-stranded DNA separates into single strands, and the cycle is repeated.

In the early versions of the PCR technique, the polymerase was destroyed by the high temperature, and new polymerase had to be added for each cycle. However, it was dis-

[1] The flash of insight didn't take long, but at least six months of hard work were needed before Mullis and his colleagues could convert the idea to reality.

[2] Short segments of single-stranded DNA.

covered that polymerase from the bacterium Thermus aquaticus would withstand the high temperature. (Thermus aquaticus lives in hot springs.) This discovery greatly simplified the PCR technique. The temperature could merely be cycled between the high and low temperatures, and with each cycle, the population of the target segment doubled, concentrations of primers, deoxynucleotides and polymerase being continuously present.

After a few cycles of the PCR reaction, copies of copies begin to predominate over copies of the original genomic DNA. These copies of copies have a standard length, always beginning on one strand with the start primer, and ending on that strand with the complement of the stop primer.

8.2 Bioinformation technology and artificial life

The merging of information technology and biotechnology

Information technology and biology are today the two most rapidly developing fields of science. Interestingly, these two fields seem to be merging, each gaining inspiration and help from the other. For example, computer scientists designing both hardware and software are gaining inspiration from physiological studies of the mechanism of the brain; and conversely, neurophysiologists are aided by insights from the field of artificial intelligence. Designers of integrated circuits wish to prolong the period of validity of Moore's law; but they are rapidly approaching physical barriers which will set limits to the miniaturization of conventional transistors and integrated circuits. They gain inspiration from biology, where the language of molecular complementarity and the principle of autoassembly seem to offer hope that molecular switches and self-assembled integrated circuits may one day be constructed.

Geneticists, molecular biologists, biochemists and crystallographers have now obtained so much information about the amino acid sequences and structures of proteins and about the nucleotide sequences in genomes that the full power of modern information technology is needed to store and to analyze this information. Computer scientists, for their part, turn to evolutionary genetics for new and radical methods of developing both software and hardware - genetic algorithms and simulated evolution.

Self-assembly of supramolecular structures; Nanoscience

In previous chapters, we saw that the language of molecular complementarity (the "lock and key" fitting discovered by Paul Ehrlich) is the chief mechanism by which information is stored and transferred in biological systems. Biological molecules have physical shapes and patterns of excess charge[3] which are recognized by complementary molecules because they fit together, just as a key fits the shape of a lock. Examples of biological "lock and key" fitting are the fit between the substrate of an enzyme and the enzyme's active site,

[3] They also have patterns of polarizable groups and reactive groups, and these patterns can also play a role in recognition.

the recognition of an antigen by its specific antibody, the specificity of base pairs in DNA and RNA, and the autoassembly of structures such as viruses and subcellular organelles.

One of the best studied examples of autoassembly through the mechanism of molecular complementarity is the tobacco mosaic virus. The assembled virus has a cylindrical form about 300 nm long (1 nm = 1 nanometer = 10^{-9} meters = 10 Ångstroms), with a width of 18 nm. The cylindrically shaped virus is formed from about 2000 identical protein molecules. These form a package around an RNA molecule with a length of approximately 6400 nucleotides. The tobacco mosaic virus can be decomposed into its constituent molecules in vitro, and the protein and RNA can be separated and put into separate bottles, as was discussed in Chapter 4.

If, at a later time, one mixes the protein and RNA molecules together in solution, they spontaneously assemble themselves into new infective tobacco mosaic virus particles. The mechanism for this spontaneous autoassembly is a random motion of the molecules through the solvent until they approach each other in such a way that a fit is formed. When two molecules fit closely together, with their physical contours matching, and with complementary patterns of excess charge also matching, the Gibbs free energy of the total system is minimized. Thus the self-assembly of matching components proceeds spontaneously, just as every other chemical reaction proceeds spontaneously when the difference in Gibbs free energy between the products and reactants is negative. The process of autoassembly is analogous to crystallization, except that the structure formed is more complex than an ordinary crystal.

A second very well-studied example of biological autoassembly is the spontaneous formation of bilayer membranes when phospholipid molecules are shaken together in water. Each phospholipid molecule has a small polar (hydrophilic) head, and a long nonpolar (hydrophobic) tail. The polar head is hydrophilic - water-loving - because it has large excess charges with which water can form hydrogen bonds. By contrast, the non-polar tail of a phospholipid molecule has no appreciable excess charges. The tail is hydrophobic - it hates water - because to fit into the water structure it has to break many hydrogen bonds to make a hole for itself, but it cannot pay for these broken bonds by forming new hydrogen bonds with water.

There is a special configuration of the system of water and phospholipid molecules which has a very low Gibbs free energy - the lipid bilayer. In this configuration, all the hydrophilic polar heads are in contact with water, while the hydrophobic nonpolar tails are in the interior of the double membrane, away from the water, and in close contact with each other, thus maximizing their mutual Van der Waals attractions. (The basic structure of biological membranes is the lipid bilayer just described, but there are also other components, such as membrane-bound proteins, caveolae, and ion pores.)

The mechanism of self-organization of supramolecular structures is one of the most important universal mechanisms of biology. Chemical reactions take place spontaneously when the change in Gibbs free energy produced by the reaction is negative, i.e., chemical reactions take place in such a direction that the entropy of the universe increases. When spontaneous chemical reactions take place, the universe moves from a less probable configuration to a more probable one. The same principle controls the motion of larger

systems, where molecules arrange themselves spontaneously to form supramolecular structures. Self-assembling collections of molecules move in such a way as to minimize their Gibbs free energy, thus maximizing the entropy of the universe.

Biological structures of all kinds are formed spontaneously from their components because assembly information is written onto their joining surfaces in the form of complementary surface contours and complementary patterns of excess charge[4]. Matching pieces fit together, and the Gibbs free energy of the system is minimized. Virtually every structure observed in biology is formed in this way - by a process analogous to crystallization, except that biological structures can be far more complex than ordinary crystals.

Researchers in microelectronics, inspired by the self-assembly of biological structures, dream of using the same principles to generate self-organizing integrated circuits with features so small as to approach molecular dimensions. As we mentioned in Chapter 7, the speed of a computing operation is limited by the time that it takes an electrical signal (moving at approximately the speed of light) to traverse a processing unit. The desire to produce ever greater computation speeds as well as ever greater memory densities, motivates the computer industry's drive towards ultraminiaturization.

Currently the fineness of detail in integrated circuits is limited by diffraction effects caused by the finite wavelength of the light used to project an image of the circuit onto a layer of photoresist covering the chip where the circuit is being built up. For this reason, there is now very active research on photolithography using light sources with extremely short wavelengths, in the deep ultraviolet, or even X-ray sources, synchrotron radiation, or electron beams. The aim of this research is to produce integrated circuits whose feature size is in the nanometer range - smaller than 100 nm. In addition to these efforts to create nanocircuits by "top down" methods, intensive research is also being conducted on "bottom up" synthesis, using principles inspired by biological self-assembly. The hope to make use of "the spontaneous association of molecules, under equilibrium conditions, into stable, structurally well-defined aggregates, joined by non-covalent bonds"[5]

The Nobel Laureate Belgian chemist J.-M. Lehn pioneered the field of supramolecular chemistry by showing that it is possible to build nanoscale structures of his own design. Lehn and his coworkers at the University of Strasbourg used positively-charged metal ions as a kind of glue to join larger structural units at points where the large units exhibited excess negative charges. Lehn predicts that the supramolecular chemistry of the future will follow the same principles of self-organization which underlie the growth of biological structures, but with a greatly expanded repertory, making use of elements (such as silicon) that are not common in carbon-based biological systems.

Other workers in nanotechnology have concentrated on the self-assembly of two-dimensional structures at water-air interfaces. For example, Thomas Bjørnholm, working at the University of Copenhagen, has shown that a nanoscale wire can be assembled spontaneously at a water-air interface, using metal atoms complexed with DNA and a DNA template. The use of a two-dimensional template to reproduce a nanostructure can be thought of as "mi-

[4] Patterns of reactive or polarizable groups also play a role.

[5] G.M. Whiteside et al., Science, **254**, 1312-1314, (1991).

croprinting". One can also think of self-assembly at surfaces as the two-dimensional version of the one-dimensional copying process by which a new DNA or RNA strand assembles itself spontaneously, guided by the complementary strand.

In 1981, Gerd Binning and Heinrich Rohrer of IBM's Research Center in Switzerland announced their invention of the scanning tunneling microscope. The new microscope's resolution was so great that single atoms could be observed. The scanning tunneling microscope consists of a supersharp conducting tip, which is brought near enough to a surface so that quantum mechanical tunneling of electrons can take place between tip and surface when a small voltage is applied. The distance between the supersharp tip and the surface is controlled by means of a piezoelectric crystal. As the tip is moved along the surface, its distance from the surface (and hence the tunneling current) is kept constant by applying a voltage to the piezoelectric crystal, and this voltage as a function of position gives an image of the surface.

Variations on the scanning tunneling microscope allow single atoms to be deposited or manipulated on a surface. Thus there is a hope that nanoscale circuit templates can be constructed by direct manipulation of atoms and molecules, and that the circuits can afterwards be reproduced using autoassembly mechanisms.

The scanning tunneling microscope makes use of a quantum mechanical effect: Electrons exhibit wavelike properties, and can tunnel small distances into regions of negative kinetic energy - regions which would be forbidden to them by classical mechanics. In general it is true that for circuit elements with feature sizes in the nanometer range, quantum effects become important. For conventional integrated circuits, the quantum effects which are associated with this size-range would be a nuisance, but workers in nanotechnology hope to design integrated circuits which specifically make use of these quantum effects.

Molecular switches; bacteriorhodopsin

The purple, salt-loving archaebacterium Halobacterium halobium (recently renamed Halobacterium salinarum) possesses one of the simplest structures that is able to perform photosynthesis. The purple membrane subtraction of this bacterium's cytoplasmic membrane contains only two kinds of molecules - lipids and bacteriorhodopsin. Nevertheless, this simple structure is able to trap the energy of a photon from the sun and to convert it into chemical energy.

The remarkable purple membrane of Halobacterium has been studied in detail by Walter Stoeckenius, D. Osterhelt[6], Lajos Keszthelyi and others.

It can be decomposed into its constituent molecules. The lipids from the membrane and the bacteriorhodopsin can be separated from each other and put into different bottles. At a later time, the two bottles can be taken from the laboratory shelf, and their contents can be shaken together in water. The result is the spontaneous formation of tiny vesicles of purple membrane.

[6] D. Osterhelt and Walter Stoeckenius, Nature New Biol. **233**, 149-152 (1971); D. Osterhelt et al., Quart. Rev. Biophys. **24**, 425-478 (1991); W. Stoeckenius and R. Bogomolni, Ann. Rev. Biochem. **52**, 587-616 (1982).

In the self-organized two-component vesicles, the membrane-bound protein bacteriorhodopsin is always correctly oriented, just as it would be in the purple membrane of a living Halobacterium. When the vesicles are illuminated, bacteriorhodopsin absorbs H^+ ions from the water on the inside, and releases them outside.

Bacteriorhodopsin consists of a chain of 224 amino acids, linked to the retinal chromophore. The amino acids are arranged in 7 helical segments, each of which spans the purple membrane, and these are joined on the membrane surface by short nonhelical segments of the chain. The chromophore is in the middle of the membrane, surrounded by a-helical segments. When the chromophore is illuminated, its color is temporarily bleached, and it undergoes a cis-trans isomerization which disrupts the hydrogen-bonding network of the protein. The result is that a proton is released on the outside of the membrane. Later, a proton is absorbed from the water in the interior of the membrane vesicle, the hydrogen-bonding system of the protein is reestablished, and both the protein and the chromophore return to their original conformations. In this way, bacteriorhodopsin functions as a proton pump. It uses the energy of photons to transport H^+ ions across the membrane, from the inside to the outside, against the electrochemical gradient. In the living Halobacterium, this H^+ concentration difference would be used to drive the synthesis of the high-energy phosphate bond of adenosine triphosphate (ATP), the inward passage of H^+ through other parts of the cytoplasmic membrane being coupled to the reaction $ADP + P_i \rightarrow ATP$ by membrane-bound reversible ATPase.

Bacteriorhodopsin is interesting as a component of one of the simplest known photosynthetic systems, and because of its possible relationship to the evolution of the eye (as was discussed in Chapter 3). In addition, researchers like Lajos Keszthelyi at the Institute of Biophysics of the Hungarian Academy of Sciences in Szeged are excited about the possible use of bacteriorhodopsin in optical computer memories[7]. Arrays of oriented and partially dehydrated bacteriorhodopsin molecules in a plastic matrix can be used to construct both 2-dimensional and 3-dimensional optical memories using the reversible color changes of the molecule. J. Chen and coworkers[8] have recently constructed a prototype 3-dimensional optical memory by orienting the proteins and afterwards polymerizing the solvent into a solid polyacrylamide matrix. Bacteriorhodopsin has extraordinary stability, and can tolerate as many as a million optical switching operations without damage.

Neural networks, biological and artificial

In 1943, W. McCulloch and W. Pitts published a paper entitled *A Logical Calculus of the Ideas Immanent in Nervous Activity*. In this pioneering paper, they proposed the idea of a Threshold Logic Unit (TLU), which they visualized not only as a model of the way in which neurons function in the brain but also as a possible subunit for artificial systems which might be constructed to perform learning and pattern-recognition tasks. Problems involving learning, generalization, pattern recognition and noisy data are easily handled

[7] A. Der and L. Keszthelyi, editors, Bioelectronic Applications of Photochromic Pigments, IOS Press, Amsterdam, Netherlands, (2001).

[8] J. Chen et al., Biosystems **35**, 145-151 (1995).

by the brains of humans and animals, but computers of the conventional von Neumann type find such tasks especially difficult.

Conventional computers consist of a memory and one or more central processing units (CPUs). Data and instructions are repeatedly transferred from the memory to the CPUs, where the data is processed and returned to the memory. The repeated performance of many such cycles requires a long and detailed program, as well as high-quality data. Thus conventional computers, despite their great speed and power, lack the robustness, intuition, learning powers and powers of generalization which characterize biological neural networks. In the 1950's, following the suggestions of McCulloch and Pitts, and inspired by the growing knowledge of brain structure and function which was being gathered by histologists and neurophysiologists, computer scientists began to construct artificial neural networks - massively parallel arrays of TLU's.

The analogy between a TLU and a neuron can be seen by comparing Figure 5.2, which shows a neuron, with Figure 8.1, which shows a TLU. As we saw in Chapter 5, a neuron is a specialized cell consisting of a cell body (*soma*) from which an extremely long, tubelike fiber called an *axon* grows. The axon is analogous to the output channel of a TLU. From the soma, a number of slightly shorter, rootlike extensions called *dendrites* also grow. The dendrites are analogous to the input channels of a TLU.

In a biological neural network, branches from the axon of a neuron are connected to the dendrites of many other neurons; and at the points of connection there are small, knoblike structures called synapses. As was discussed in Chapter 5, the "firing" of a neuron sends a wave of depolarization out along its axon. When the pulselike electrical and chemical disturbance associated with the wave of depolarization (the action potential) reaches a synapse, where the axon is connected with another neuron, transmitter molecules are released into the post-synaptic cleft. The neurotransmitter molecules travel across the post-synaptic cleft to receptors on a dendrite of the next neuron in the net, where they are bound to receptors. There are many kinds of neurotransmitter molecules, some of which tend to make the firing of the next neuron more probable, and others which tend to inhibit its firing. When the neurotransmitter molecules are bound to the receptors, they cause a change in the dendritic membrane potential, either increasing or decreasing its polarization. The post-synaptic potentials from the dendrites are propagated to the soma; and if their sum exceeds a threshold value, the neuron fires. The subtlety of biological neural networks derives from the fact that there are many kinds of neurotransmitters and synapses, and from the fact that synapses are modified by their past history.

Turning to Figure 8.1, we can compare the biological neuron with the Threshold Logic Unit of McCulloch and Pitts. Like the neuron, the TLU has many input channels. To each of the N channels there is assigned a weight, $w_1, w_2, ..., w_N$. The weights can be changed; and the set of weights gives the TLU its memory and learning capabilities. Modification of weights in the TLU is analogous to the modification of synapses in a neuron, depending on their history. In the most simple type of TLU, the input signals are either 0 or 1. These signals, multiplied by their appropriate weights, are summed, and if the sum exceeds a threshold value, θ the TLU "fires", i.e. a pulse of voltage is transmitted through the output channel to the next TLU in the artificial neural network.

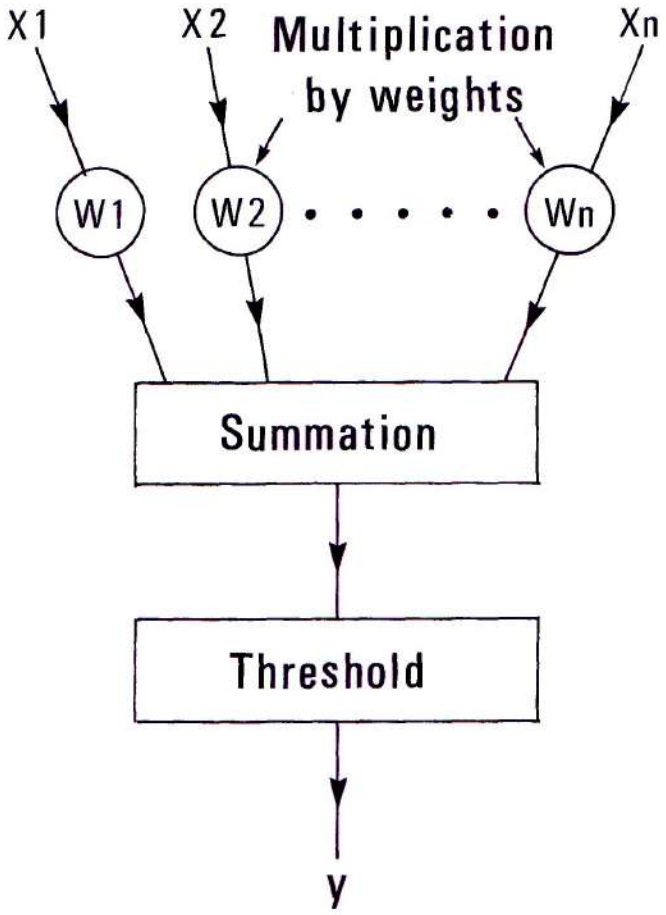

Figure 8.1: A Threshold Logic Unit (TLU) of the type proposed by McCulloch and Pitts.

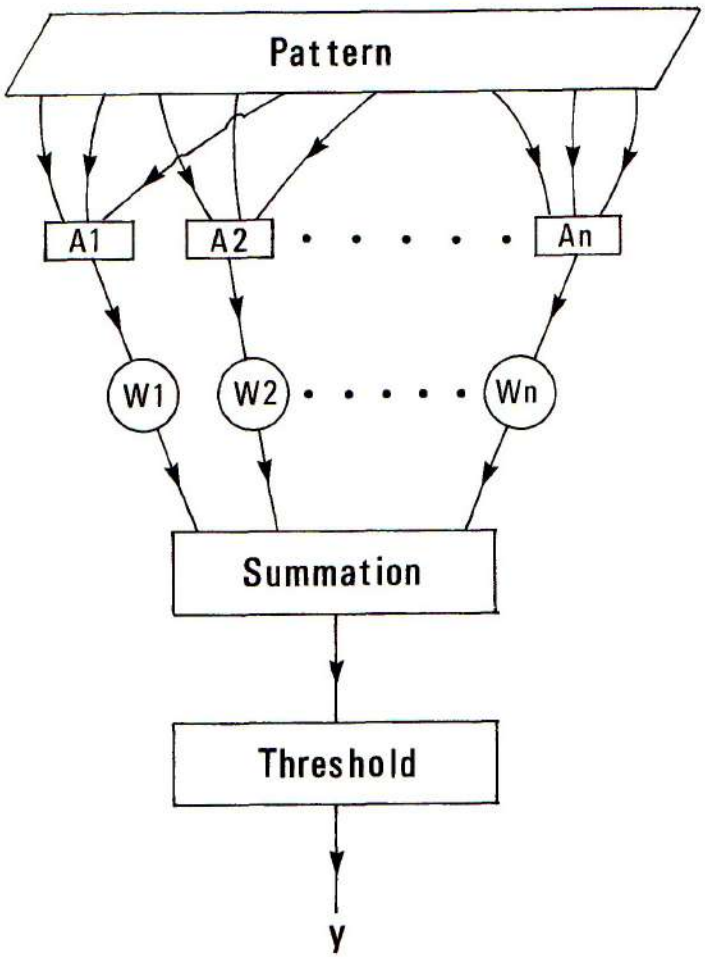

Figure 8.2: A perceptron, introduced by Rosenblatt in 1962. The perceptron is similar to a TLU, but its input is preprocessed by a set of association units (A-units). The A-units are not trained, but are assigned a fixed Boolean functionality.

Let us imagine that the input signals, $x_1, x_2, ..., x_N$ can take on the values 0 or 1. The weighted sum of the input signals will then be given by

$$a = \sum_{j=1}^{N} w_j x_j \tag{8.1}$$

The quantity a, is called the *activation*. If the activation exceeds the threshold 9, the unit "fires", i.e. it produces an output y given by

$$y = \begin{cases} 1 & \text{if} \quad a \geq \theta \\ \\ 0 & \text{if} \quad a < \theta \end{cases} \tag{8.2}$$

The decisions taken by a TLU can be given a geometrical interpretation: The input signals can be thought of as forming the components of a vector, $x = x_1, x_2, ..., X_N$, in an N-dimensional space called pattern space. The weights also form a vector, $w = w_1, w_2, ..., w_N$, in the same space. If we write an equation setting the scalar product of these two vectors equal to some constant,

$$\mathbf{w} \cdot \mathbf{x} \equiv \sum_{j=1}^{N} w_j x_j = \theta \tag{8.3}$$

then this equation defines a hyperplane in pattern space, called the *decision hyperplane*. The decision hyperplane divides pattern space into two parts - (1) input pulse patterns which will produce firing of the TLU, and (2) patterns which will not cause firing.

The position and orientation of the decision hyperplane can be changed by altering the weight vector w and/or the threshold θ. Therefore it is convenient to put the threshold and the weights on the same footing by introducing an augmented weight vector,

$$\mathbf{W} = w_1, w_2, ..., w_N, \theta \tag{8.4}$$

and an augmented input pattern vector,

$$\mathbf{X} = x_1, x_2, ..., x_N, -1 \tag{8.5}$$

In the N+l-dimensional augmented pattern space, the decision hyperplane now passes through the origin, and equation (8.3) can be rewritten in the form

$$\mathbf{W} \cdot \mathbf{X} \equiv \sum_{j=1}^{N+1} W_j X_j = 0 \tag{8.6}$$

Those input patterns for which the scalar product $\mathbf{W} \cdot \mathbf{X}$ is positive or zero will cause the unit to fire, but if the scalar product is negative, there will be no response.

If we wish to "teach" a TLU to fire when presented with a particular pattern vector $\mathbf{X}$, we can evaluate its scalar product with the current augmented weight vector $\mathbf{W}$. If this

scalar product is negative, the TLU will not fire, and therefore we know that the weight vector needs to be changed. If we replace the weight vector by

$$\mathbf{W}' = \mathbf{W} + \gamma\mathbf{X} \tag{8.7}$$

where γ is a small positive number, then the new augmented weight vector $\mathbf{W}'$ will point in a direction more nearly the same as the direction of $\mathbf{X}$. This change will be a small step in the direction of making the scalar product positive, i.e. a small step in the right direction.

Why not take a large step instead of a small one? A small step is best because there may be a whole class of input patterns to which we would like the TLU to respond by firing. If we make a large change in weights to help a particular input pattern, it may undo previous learning with respect to other patterns.

It is also possible to teach a TLU to remain silent when presented with a particular input pattern vector. To do so we evaluate the augmented scalar product $\mathbf{W} \cdot \mathbf{X}$ as before, but now, when we desire silence rather than firing, we wish the scalar product to be negative, and if it is positive, we know that the weight vector must be changed. In changing the weight vector, we can again make use of equation (8.7), but now γ must be a small negative number rather than a small positive one.

Two sets of input patterns, A and B, are said to be linearly separable if they can be separated by some decision hyperplane in pattern space. Now suppose that the four sets, A, B, C, and D, can be separated by two decision hyperplanes. We can then construct a two-layer network which will identify the class of an input signal belonging to any one of the sets, as is illustrated in Figure 8.2.

The first layer consists of two TLU's. The first TLU in this layer is taught to fire if the input pattern belongs to A or B, and to be silent if the input belongs to C or D. The second TLU is taught to fire if the input pattern belongs to A or D, and to be silent if it belongs to B or C. The second layer of the network consists of four output units which are not taught, but which are assigned a fixed Boolean functionality. The first output unit fires if the signals from the first layer are given by the vector $\mathbf{y} = \{0, 0\}$ (class A); the second fires if $\mathbf{y} = \{0, 1\}$ (class B), the third if $\mathbf{y} = \{1, 0\}$ (class C), and the fourth if $\mathbf{y} = \{1, 1\}$ (class D). Thus the simple two-layer network shown in Figure 8.2 functions as a *classifier*. The output units in the second layer are analogous to the "grandmother's face cells" whose existence in the visual cortex is postulated by neurophysiologists. These cells will fire if and only if the retina is stimulated with a particular class of patterns.

This very brief glance at artificial neural networks does not do justice to the high degree of sophistication which network architecture and training algorithms have achieved during the last two decades. However, the suggestions for further reading at the end of this chapter may help to give the reader an impression of the wide range of problems to which these networks are now being applied.

Besides being useful for computations requiring pattern recognition, learning, generalization, intuition, and robustness in the face of noisy data, artificial neural networks are important because of the light which they throw on the mechanism of brain function. For

example, one can compare the classifier network shown in Figure 8.2 with the discoveries of Kuffler, Hubel and Wessel concerning pattern abstraction in the mammalian retina and visual cortex (Chapter 5).

Genetic algorithms

Genetic algorithms represent a second approach to machine learning and to computational problems involving optimization. Like neural network computation, this alternative approach has been inspired by biology, and it has also been inspired by the Darwinian concept of natural selection. In a genetic algorithm, the hardware is that of a conventional computer; but the software creates a population and allows it to evolve in a manner closely analogous to biological evolution.

One of the most important pioneers of genetic algorithms was John Henry Holland (1929-). After attending MIT, where he was influenced by Norbert Wiener, Holland worked for IBM, helping to develop the 701. He then continued his studies at the University of Michigan, obtaining the first Ph.D. in computer science ever granted in America. Between 1962 and 1965, Holland taught a graduate course at Michigan called "Theory of Adaptive Systems". His pioneering course became almost a cult, and together with his enthusiastic students he applied the genetic algorithm approach to a great variety of computational problems. One of Holland's students, David Goldberg, even applied a genetic algorithm program to the problem of allocating natural gas resources.

The programs developed by Holland and his students were modelled after the natural biological processes of reproduction, mutation, selection and evolution. In biology, the information passed between generations is contained in chromosomes - long strands of DNA where the genetic message is written in a four-letter language, the letters being adenine, thymine, guanine and cytosine. Analogously, in a genetic algorithm, the information is coded in a long string, but instead of a four-letter language, the code is binary: The chromosome-analogue is a long string of 0's and 1's, i.e., a long binary string. One starts with a population that has sufficient diversity so that natural selection can act.

The genotypes are then translated into phenotypes. In other words, the information contained in the long binary string (analogous to the genotype of each individual) corresponds to an entity, the phenotype, whose fitness for survival can be evaluated. The mapping from genotype to phenotype must be such that very small changes in the binary string will not produce radically different phenotypes. Prom the initial population, the most promising individuals are selected to be the parents of the next generation, and of these, the fittest are allowed produce the largest number of offspring. Before reproduction takes place, however, random mutations and chromosome crossing can occur. For example, in chromosome crossing, the chromosomes of two individuals are broken after the nth binary digit, and two new chromosomes are formed, one with the head of the first old chromosome and the tail of the second, and another with the head of the second and the tail of the first. This process is analogous to the biological crossings which allowed Thomas Hunt Morgan and his "fly squad" to map the positions of genes on the chromosomes of fruit flies, while the mutations are analogous to those studied by Hugo de Vries and Hermann

J. Muller.

After the new generation has been produced, the genetic algorithm advances the time parameter by a step, and the whole process is repeated: The phenotypes of the new generation are evaluated and the fittest selected to be parents of the next generation; mutation and crossings occur; and then fitness-proportional reproduction. Like neural networks, genetic algorithms are the subject of intensive research, and evolutionary computation is a rapidly growing field.

Evolutionary methods have been applied not only to software, but also to hardware. Some of the circuits designed in this way defy analysis using conventional techniques - and yet they work astonishingly well.

Artificial life

As Aristotle pointed out, it is difficult to define the precise border between life and nonlife. It is equally difficult to give a precise definition of artificial life. Of course the term means "life produced by humans rather than by nature", but what is life? Is self-replication the only criterion? The phrase "produced by humans" also presents difficulties. Humans have played a role in creating domestic species of animals and plants. Can cows, dogs, and high-yield wheat varieties be called "artificial life" ? In one sense, they can. These species and varieties certainly would not have existed without human intervention.

We come nearer to what most people might call "artificial life" when we take parts of existing organisms and recombine them in novel ways, using the techniques of biotechnology. For example, Steen Willadsen[9], working at the Animal Research Station, Cambridge England, was able to construct chimeras by operating under a microscope on embryos at the eight-cell stage. The zona pelucida is a transparent shell that surrounds the cells of the embryo. Willadsen was able to cut open the zona pelucida, to remove the cells inside, and to insert a cell from a sheep embryo together with one from a goat embryo. The chimeras which he made in this way were able to grow to be adults, and when examined, their cells proved to be a mosaic, some cells carrying the sheep genome while others carried the genome of a goat. By the way, Willadsen did not create his chimeras in order to produce better animals for agriculture. He was interested in the scientifically exciting problem of morphogenesis: How is the information of the genome translated into the morphology of the growing embryo?

Human genes are now routinely introduced into embryos of farm animals, such as pigs or sheep. The genes are introduced into regulatory sequences which cause expression in mammary tissues, and the adult animals produce milk containing human proteins. Many medically valuable proteins are made in this way. Examples include human blood-clotting factors, interleukin-2 (a protein which stimulates T-lymphocytes), collagen and fibrinogen (used to treat burns), human fertility hormones, human hemoglobin, and human serum albumin.

[9] Willadsen is famous for having made the first verified and reproducible clone of a mammal. In 1984 he made two genetically identical lambs from early sheep embryo cells.

Transgenic plants and animals in which the genes of two or more species are inherited in a stable Mendelian way have become commonplace in modern laboratory environments, and, for better or for worse, they are also becoming increasingly common in the external global environment. These new species might, with some justification, be called "artificial life".

In discussing the origin of life in Chapter 3, we mentioned that a long period of molecular evolution probably preceded the evolution of cells. In the early 1970's, S. Spiegelman performed a series of experiments in which he demonstrated that artificial molecular evolution can be made to take place in vitro. Spiegelman prepared a large number of test tubes in which RNA replication could take place. The aqueous solution in each of the test tubes consisted of RNA replicase, ATP, UTP (uracil triphosphate), GTP (guanine triphosphate), CTP (cytosine triphosphate) and buffer. He then introduced RNA from a bacteriophage into the first test tube. After a predetermined interval of time, during which replication took place, Spiegelman transferred a drop of solution from the first test tube to a new tube, uncontaminated with RNA. Once again, replication began and after an interval a drop was transferred to a third test tube. Spiegelman repeated this procedure several hundred times, and at the end he was able to demonstrate that the RNA in the final tube differed from the initial sample, and that it replicated faster than the initial sample. The RNA had evolved by the classical Darwinian mechanisms of mutation and natural selection. Mistakes in copying had produced mutant RNA strands which competed for the supply of energy-rich precursor molecules (ATP, UTP, GTP and CTP). The most rapidly-reproducing mutants survived. Was Spiegelman's experiment merely a simulation of an early stage of biological evolution? Or was evolution of an extremely primitive life-form actually taking place in his test tubes?

G.F. Joyce, D.P. Bartel and others have performed experiments in which strands of RNA with specific catalytic activity (ribozymes) have been made to evolve artificially from randomly coded starting populations of RNA. In these experiments, starting populations of 1013 to 1015 randomly coded RNA molecules are tested for the desired catalytic activity, and the most successful molecules are then chosen as parents for the next generation. The selected molecules are replicated many times, but errors (mutations) sometimes occur in the replication. The new population is once again tested for catalytic activity, and the process is repeated. The fact that artificial evolution of ribozymes is possible can perhaps be interpreted as supporting the "RNA world" hypothesis, i.e. the hypothesis that RNA preceded DNA and proteins in the early history of terrestrial life.

In Chapter 4 we mentioned that John von Neumann speculated on the possibility of constructing artificial self-reproducing automata. In the early 1940's, a period when there was much discussion of the Universal Turing Machine, he became interested in constructing a mathematical model of the requirements for self-reproduction. Besides the Turing machine, another source of his inspiration was the paper by Warren McCulloch and Walter Pitts entitled *A logical calculus of the ideas immanent in nervous activity*, which von Neumann read in 1943. In his first attempt (the kinematic model), he imagined an extremely large and complex automaton, floating on a lake which contained its component parts.

Von Neumann's imaginary self-reproducing automaton consisted of four units, A, B, C

and D. Unit A was a sort of factory, which gathered component parts from the surrounding lake and assembled them according to instructions which it received from other units. Unit B was a copying unit, which reproduced sets of instructions. Unit C was a control apparatus, similar to a computer. Finally D was a long string of instructions, analogous to the "tape" in the Turing machine described in Chapter 7. In von Neumann's kinematic automaton, the instructions were coded as a long binary number. The presence of what he called a "girder" at a given position corresponded to 1, while its absence corresponded to 0. In von Neumann's model, the automaton completed the assembly of its offspring by injecting its progeny with the duplicated instruction tape, thus making the new automaton both functional and fertile.

In presenting his kinematic model at the Hixton Symposium (organized by Linus Pauling in the late 1940's), von Neumann remarked that "...it is clear that the instruction [tape] is roughly effecting the function of a gene. It is also clear that the copying mechanism B performs the fundamental act of reproduction, the duplication of the genetic material, which is clearly the fundamental operation in the multiplication of living cells. It is also easy to see how arbitrary alterations of the system...can exhibit certain traits which appear in connection with mutation, lethality as a rule, but with a possibility of continuing reproduction with a modification of traits."

It is very much to von Neumann's credit that his kinematic model (which he invented several years before Crick and Watson published their DNA structure) was organized in much the same way that we now know the reproductive apparatus of a cell to be organized. Nevertheless he was dissatisfied with the model because his automaton contained too many "black boxes". There were too many parts which were supposed to have certain functions, but for which it seemed very difficult to propose detailed mechanisms by which the functions could be carried out. His kinematic model seemed very far from anything which could actually be built[10].

Von Neumann discussed these problems with his close friend, the Polish-American mathematician Stanislaw Ulam, who had for a long time been interested in the concept of self-replicating automata. When presented with the black box difficulty, Ulam suggested that the whole picture of an automaton floating on a lake containing its parts should be discarded. He proposed instead a model which later came to be known as the Cellular Automaton Model. In Ulam's model, the self-reproducing automaton lives in a very special space. For example, the space might resemble an infinite checkerboard, each square would constitute a multi-state cell. The state of each cell in a particular time interval is governed by the states of its near neighbors in the preceding time interval according to relatively simple laws. The automaton would then consist of a special configuration of cell states, and its reproduction would correspond to production of a similar configuration of cell states in

[10] Von Neumann's kinematic automaton was taken seriously by the Mission IV Group, part of a ten-week program sponsored by NASA in 1980 to study the possible use of advanced automation and robotic devices in space exploration. The group, headed by Richard Laing, proposed plans for self-reproducing factories, designed to function on the surface of the moon or the surfaces of other planets. Like von Neumann's kinetic automaton, to which they owed much, these plans seemed very far from anything that could actually be constructed.

a neighboring region of the cell lattice.

Von Neumann liked Ulam's idea, and he began to work in that direction. However, he wished his self-replicating automaton to be able to function as a universal Turing machine, and therefore the plans which he produced were excessively complicated. In fact, von Neumann believed complexity to be a necessary requirement for self-reproduction. In his model, the cells in the lattice were able to have 29 different states, and the automaton consisted of a configuration involving hundreds of thousands of cells. Von Neumann's manuscript on the subject became longer and longer, and he did not complete it before his early death from prostate cancer in 1957. The name "cellular automaton" was coined by Arthur Burks, who edited von Neumann's posthumous papers on the theory of automata.

Arthur Burks had written a Ph.D. thesis in philosophy on the work of the nineteenth century thinker Charles Sanders Pierce, who is today considered to be one of the founders of semiotics[11]. He then studied electrical engineering at the Moore School in Philadelphia, where he participated in the construction of ENIAC, one of the first general purpose electronic digital computers, and where he also met John von Neumann. He worked with von Neumann on the construction of a new computer, and later Burks became the leader of the Logic of Computers Group at the University of Michigan. One of Burks' students at Michigan was John Holland, the pioneer of genetic algorithms. Another student of Burks, E.F. Codd, was able to design a self-replicating automaton of the von Neumann type using a cellular automaton system with only 8 states (as compared with von Neumann's 29). For many years, enthusiastic graduate students at the Michigan group continued to do important research on the relationships between information, logic, complexity and biology.

Meanwhile, in 1968, the mathematician John Horton Conway, working in England at Cambridge University, invented a simple game which greatly increased the popularity of the cellular automaton concept. Conway's game, which he called "Life", was played on an infinite checker-board-like lattice of cells, each cell having only two states, "alive" or "dead". The rules which Conway proposed are as follows: "If a cell on the checkerboard is alive, it will survive in the next time step (generation) if there are either two or three neighbors also alive. It will die of overcrowding if there are more than three live neighbors, and it will die of exposure if there are fewer than two. If a cell on the checkerboard is dead, it will remain dead in the next generation unless exactly three of its eight neighbors is alive. In that case, the cell will be 'born' in the next generation".

Originally Conway's Life game was played by himself and by his colleagues at Cambridge University's mathematics department in their common room: At first the game was played on table tops at tea time. Later it spilled over from the tables to the floor, and tea time began to extend: far into the afternoons. Finally, wishing to convert a wider audience to his game, Conway submitted it to Martin Gardner, who wrote a popular column on "Mathematical Games" for the Scientific American. In this way Life spread to MIT's Artificial Intelligence Laboratory, where it created such interest that the MIT group designed a small computer specifically dedicated to rapidly implementing Life's rules.

[11] Semiotics is defined as the study of signs (see Appendix 2).

The reason for the excitement about Conway's Life game was that it seemed capable of generating extremely complex patterns, starting from relatively simple configurations and using only its simple rules. Ed Fredkin, the director of MIT's Artificial Intelligence Laboratory, became enthusiastic about cellular automata because they seemed to offer a model for the way in which complex phenomena can emerge from the laws of nature, which are after all very simple. In 1982, Fredkin (who was independently wealthy because of a successful computer company which he had founded) organized a conference on cellular automata on his private island in the Caribbean. The conference is notable because one of the participants was a young mathematical genius named Stephen Wolfram, who was destined to refine the concept of cellular automata and to become one of the leading theoreticians in the field[12].

One of Wolfram's important contributions was to explore exhaustively the possibilities of 1-dimensional cellular automata. No one before him had looked at 1-dimensional CA's, but in fact they had two great advantages: The first of these advantages was simplicity, which allowed Wolfram to explore and classify the possible rule sets. Wolfram classified the rule sets into 4 categories, according to the degree of complexity which they generated. The second advantage was that the configurations of the system in successive generations could be placed under one another to form an easily-surveyed 2-dimensional visual display. Some of the patterns generated in this way were strongly similar to the patterns of pigmentation on the shells of certain molluscs. The strong resemblance seemed to suggest that Wolfram's 1-dimensional cellular automata might yield insights into the mechanism by which the pigment patterns are generated.

In general, cellular automata seemed to be promising models for gaining insight into the fascinating and highly important biological problem of morphogenesis: How does the fertilized egg translate the information on the genome into the morphology of the growing embryo, ending finally with the enormously complex morphology of a fully developed and fully differentiated multicellular animal? Our understanding of this amazing process is as yet very limited, but there is evidence that as the embryo of a multicellular animal develops, cells change their state in response to the states of neighboring cells. In the growing embryo, the "state" of a cell means the way in which it is differentiated, i.e., which genes are turned on and which off - which information on the genome is available for reading, and which segments are blocked. Neighboring cells signal to each other by means of chemical messengers[13]. Clearly there is a close analogy between the way complex patterns develop in a cellular automaton, as neighboring cells influence each other and change their states according to relatively simple rules, and the way in which the complex morphology of a multicellular animal develops in the growing embryo.

Conway's Life game attracted another very important worker to the field of cellular automata: In 1971, Christopher Langton was working as a computer programmer in the Stanley Cobb Laboratory for Psychiatric Research at Massachusetts General Hospital.

[12] As many readers probably know, Stephen Wolfram was also destined to become a millionaire by inventing the elegant symbol-manipulating program system, Mathematica.

[13] We can recall the case of slime mold cells which signal to each other by means of the chemical messenger, cyclic AMP (Chapter 3).

When colleagues from MIT brought to the laboratory a program for executing Life, Langton was immediately interested. He recalls "It was the first hint that there was a distinction between the hardware and the behavior which it would support... You had the feeling that there was something very deep here in this little artificial universe and its evolution through time. [At the lab] we had a lot of discussions about whether the program could be open ended - could you have a universe in which life could evolve?"

Later, at the University of Arizona, Langton read a book describing von Neumann's theoretical work on automata. He contacted Arthur Burks, von Neumann's editor, who told him that no self-replicating automaton had actually been implemented, although E.F. Codd had proposed a simplified plan with only 8 states instead of 29. Burks suggested to Langton that he should start by reading Codd's book.

When Langton studied Codd's work, he realized that part of the problem was that both von Neumann and Codd had demanded that the self-reproducing automaton should be able to function as a universal Turing machine, i.e., as a universal computer. When Langton dropped this demand (which he considered to be more related to mathematics than to biology) he was able to construct a relatively simple self-reproducing configuration in an 8-state 2-dimensional lattice of CA cells. As they reproduced themselves, Langton's loop-like cellular automata filled the lattice of cells in a manner reminiscent of a growing coral reef, with actively reproducing loops on the surface of the filled area, and "dead" (nonreproducing) loops in the center.

Langton continued to work with cellular automata as a graduate student at Arthur Burks' Logic of Computers Group at Michigan. His second important contribution to the field was an extension of Wolfram's classification of rule sets for cellular automata. Langton introduced a parameter A to characterize various sets of rules according to the type of behavior which they generated. Rule sets with a value near to the optimum ($\lambda = 0.273$) generated complexity similar to that found in biological systems. This value of Langton's λ parameter corresponded to a borderline region between periodicity and chaos.

After obtaining a Ph.D. from Burks' Michigan group, Christopher Langton moved to the Center for Nonlinear Studies at Los Alamos, New Mexico, where in 1987 he organized an "Interdisciplinary Workshop on the Synthesis and Simulation of Living Systems" - the first conference on artificial life ever held. Among the participants were Richard Dawkins, Astrid Lindenmayer, John Holland, and Richard Laing. The noted Oxford biologist and author Richard Dawkins was interested in the field because he had written a computer program for simulating and teaching evolution. Astrid Lindenmayer and her coworkers in Holland had written programs capable of simulating the morphogenesis of plants in an astonishingly realistic way. As was mentioned above, John Holland pioneered the development of genetic algorithms, while Richard Laing was the leader of Nasals study to determine whether self-reproducing factories might be feasible.

Langton's announcement for the conference, which appeared in the Scientific American, stated that "Artificial life is the study of artificial systems that exhibit behavior characteristic of natural living systems...The ultimate goal is to extract the logical form of living systems. Microelectronic technology and genetic engineering will soon give us the capability to create new life *in silico* as well as *in vitro*. This capacity will present humanity with

the most far-reaching technical, theoretical, and ethical challenges it has ever confronted. The time seems appropriate for a gathering of those involved in attempts to simulate or synthesize aspects of living systems."

In the 1987 workshop on artificial life, a set of ideas which had gradually emerged during the previous decades of work on automata and simulations of living systems became formalized and crystallized: All of the participants agreed that something more than reductionism was needed to understand the phenomenon of life. This belief was not a revival of vitalism; it was instead a conviction that the abstractions of molecular biology are not in themselves sufficient. The type of abstraction found in Darwin's theory of natural selection was felt to be nearer to what was needed. The viewpoints of thermodynamics and statistical mechanics were also helpful. What was needed, it was felt, were insights into the flow of information in complex systems; and computer simulations could give us this insight. The fact that the simulations might take place in silico did not detract from their validity. The logic and laws governing complex systems and living systems were felt to be independent of the medium.

As Langton put it, "The ultimate goal of artificial life would be to create 'life' in some other medium, ideally a virtual medium where the essence of life has been abstracted from the details of its implementation in any particular model. We would like to build models that are so lifelike that they cease to become models of life and become examples of life themselves."

Most of the participants at the first conference on artificial life had until then been working independently, not aware that many other researchers shared their viewpoint. Their conviction that the logic of a system is largely independent of the medium echoes the viewpoint of the Macy Conferences on cybernetics in the 1940's, where the logic of feedback loops and control systems was studied in a wide variety of contexts, ranging from biology and anthropology to computer systems. A similar viewpoint can also be found in biosemiotics (Appendix 2), where, in the words of the Danish biologist Jesper Hoffmeyer, "the sign, rather than the molecule" is considered to be the starting point for studying life. In other words, the essential ingredient of life is information; and information can be expressed in many ways. The medium is less important than the message.

The conferences on artificial life have been repeated each year since 1987, and European conferences devoted to the new and rapidly growing field have also been organized. Langton himself moved to the Santa Fe Institute, where he became director of the institute's artificial life program and editor of a new journal, *Artificial Life*. The first three issues of the journal have been published as a book by the MIT Press, and the book presents an excellent introduction to the field.

Among the scientists who were attracted to the artificial life conferences was the biologist Thomas Ray, a graduate of Florida State University and Harvard, and an expert in the ecology of tropical rain forests. In the late 1970's, while he was working on his Harvard Ph.D., Ray happened to have a conversation with a computer expert from the MIT Artificial Intelligence Lab, who mentioned to him that computer programs can replicate. To Ray's question "How?", the AI man answered "Oh, it's trivial."

Ray continued to study tropical ecologies, but the chance conversation from his Cam-

bridge days stuck in his mind. By 1989 he had acquired an academic post at the University of Delaware, and by that time he had also become proficient in computer programming. He had followed with interest the history of computer viruses. Were these malicious creations in some sense alive? Could it be possible to make self-replicating computer programs which underwent evolution by natural selection? Ray considered John Holland's genetic algorithms to be analogous to the type of selection imposed by plant and animal breeders in agriculture. He wanted to see what would happen to populations of digital organisms that found their own criteria for natural selection - not humanly imposed goals, but self-generated and open-ended criteria growing naturally out of the requirements for survival.

Although he had a grant to study tropical ecologies, Ray neglected the project and used most of his time at the computer, hoping to generate populations of computer organisms that would evolve in an open-ended and uncontrolled way. Luckily, before starting his work in earnest, Thomas Ray consulted Christopher Langton and his colleague James Farmer at the Center for Nonlinear Studies in New Mexico. Langton and Farmer realized that Ray's project could be a very dangerous one, capable of producing computer viruses or worms far more malignant and difficult to eradicate than any the world had yet seen. They advised Ray to make use of Turing's concept of a virtual computer. Digital organisms created in such a virtual computer would be unable to live outside it. Ray adopted this plan, and began to program a virtual world in which his freely evolving digital organisms could live. He later named the system "Tierra".

Ray's Tierra was not the first computer system to aim at open-ended evolution. Steen Rasmussen, working at the Danish Technical University, had previously produced a system called "VENUS" (Virtual Evolution in a Nonstochastic Universe Simulator) which simulated the very early stages of the evolution of life on earth. However, Ray's aim was not to understand the origin of life, but instead to produce digitally something analogous to the evolutionary explosion of diversity that occurred on earth at the start of the Cambrian era. He programmed an 80-byte self-reproducing digital organism which he called "Ancestor", and placed it in Tierra, his virtual Garden of Eden.

Ray had programmed a mechanism for mutation into his system, but he doubted that he would be able to achieve an evolving population with his first attempt. As it turned out, Ray never had to program another organism. His 80-byte Ancestor reproduced and populated his virtual earth, changing under the action of mutation and natural selection in a way that astonished and delighted him.

In his freely evolving virtual zoo, Ray found parasites, and even hyperparasites, but he also found instances of altruism and symbiosis. Most astonishingly of all, when he turned off the mutations in his Eden, his organisms invented sex (using mechanisms which Ray had introduced to allow for parasitism). They had never been told about sex by their creator, but they seemed to find their own way to the Tree of Knowledge.

Thomas Ray expresses the aims of his artificial life research as follows:[14] "Everything we know about life is based on one example: Life on Earth. Everything we know about intelligence is based on one example: Human intelligence. This limited experience burdens

[14] T. Ray, http://www.hip.atr.co.jp/ ray/pubs/pubs.html

us with preconceptions, and limits our imaginations... How can we go beyond our conceptual limits, find the natural form of intelligent processes in the digital medium, and work with the medium to bring it to its full potential, rather than just imposing the world we know upon it by forcing it to run a simulation of our physics, chemistry and biology?..."

"In the carbon medium it was evolution that explored the possibilities inherent in the medium, and created the human mind. Evolution listens to the medium it is embedded in. It has the advantage of being mindless, and therefore devoid of preconceptions, and not limited by imagination." "I propose the creation of a digital nature - a system of wildlife reserves in cyberspace in the interstices between human colonizations, feeding off unused CPU-cycles and permitted a share of our bandwidth. This would be a place where evolution can spontaneously generate complex information processes, free from the demands of human engineers and market analysts telling it what the target applications are - a place for a digital Cambrian explosion of diversity and complexity..."

"It is possible that out of this digital nature, there might emerge a digital intelligence, truly rooted in the nature of the medium, rather than brutishly copied from organic nature. It would be a fundamentally alien intelligence, but one that would complement rather than duplicate our talents and abilities."

Have Thomas Ray and other "a-lifers"[15] created artificial living organisms? Or have they only produced simulations that mimic certain aspects of life? Obviously the answer to this question depends on the definition of life, and there is no commonly agreed-upon definition. Does life have to involve carbon chemistry? The a-lifers call such an assertion "carbon chauvinism". They point out that elsewhere in the universe there may exist forms of life based on other media, and their program is to find medium-independent characteristics which all forms of life must have.

In the present book, especially in Chapter 4, we have looked at the phenomenon of life from the standpoint of thermodynamics, statistical mechanics and information theory. Seen from this viewpoint, a living organism is a complex system produced by an input of thermodynamic information in the form of Gibbs free energy. This incoming information keeps the system very far away from thermodynamic equilibrium, and allows it to achieve a statistically unlikely and complex configuration. The information content of any complex (living) system is a measure of how unlikely it would be to arise by chance. With the passage of time, the entropy of the universe increases, and the almost unimaginably improbable initial configuration of the universe is converted into complex free-energy-using systems that could never have arisen by pure chance. Life maintains itself and evolves by feeding on Gibbs free energy, that is to say, by feeding on the enormous improbability of the initial conditions of the universe.

All of the forms of artificial life that we have discussed derive their complexity from the consumption of free energy. For example, Spiegelman's evolving RNA molecules feed on the Gibbs free energy of the phosphate bonds of their precursors, ATP, GTP, UTP, and CTP. This free energy is the driving force behind artificial evolution which Spiegelman observed. In his experiment, thermodynamic information in the form of high-energy phosphate bonds

[15] In this terminology, ordinary biologists are "b-lifers".

is converted into cybernetic information.

Similarly, in the polymerase chain reaction, discussed in Chapter 3, the Gibbs free energy of the phosphate bonds in the precursor molecules ATP, TTP, GTP and CTP drives the reaction. With the aid of the enzyme DNA polymerase, the soup of precursors is converted into a highly improbable configuration consisting of identical copies of the original sequence. Despite the high improbability of the resulting configuration, the entropy of the universe has increased in the copying process. The improbability of the set of copies is less than the improbability of the high energy phosphate bonds of the precursors.

The polymerase chain reaction reflects on a small scale, what happens on a much larger scale in all living organisms. Their complexity is such that they never could have originated by chance, but although their improbability is extremely great, it is less than the still greater improbability of the configurations of matter and energy from which they arose. As complex systems are produced, the entropy of the universe continually increases, i.e., the universe moves from a less probable configuration to a more probable one.

In Thomas Ray's experiments, the source of thermodynamic information is the electrical power needed to run the computer. In an important sense one might say that the digital organisms in Ray's Tierra system are living. This type of experimentation is in its infancy, but since it combines the great power of computers with the even greater power of natural selection, it is hard to see where it might end.

8.3 Molecular biology and the COVID-19 pandemic

Starting in December, 2019, and accelerating rapidly during the spring of 2020, our world has been hit by a new and extremely serious pandemic. It is caused by a caronavirus closely related to bat coronaviruses, and the disease, designated COVID-19 has a high death rate compared with seasonal influenza. As of April 1, 2020, more than 859,000 cases of COVID-19 have been reported in over 200 countries and territories, resulting in approximately 42,000 deaths. Of course the death rate is actually lower that would be calculated from the ratio 42/859=0.049, since the actual number of infected people is very much larger than the number of confirmed cases. Older people, and people with previously existing health problems are especially at risk.

The first cases of COVID-19 were noticed in the city of Wuhan, in the Hubei province of China. A cluster of cases centered on the Hunan Seafood Wholesale Market, and the outbreak is thought to have been a case where a virus has been transmitted from an animal host to humans.

The World Health Organization recognized the outbreak as being a Public Health Emergency of International Concern on January 30, 2020. Later, on March 11, 2020, WHO declared it to be a pandemic.

Governments around the world have reacted to the pandemic by closing borders, closing schools, universities, restaurants, barber shops, bars, sports events, and nonessential economic activities of all sorts, also requiring people to stay at home, and requesting them to practice "social distancing", i.e. staying at least 2 meters from all others, even fam-

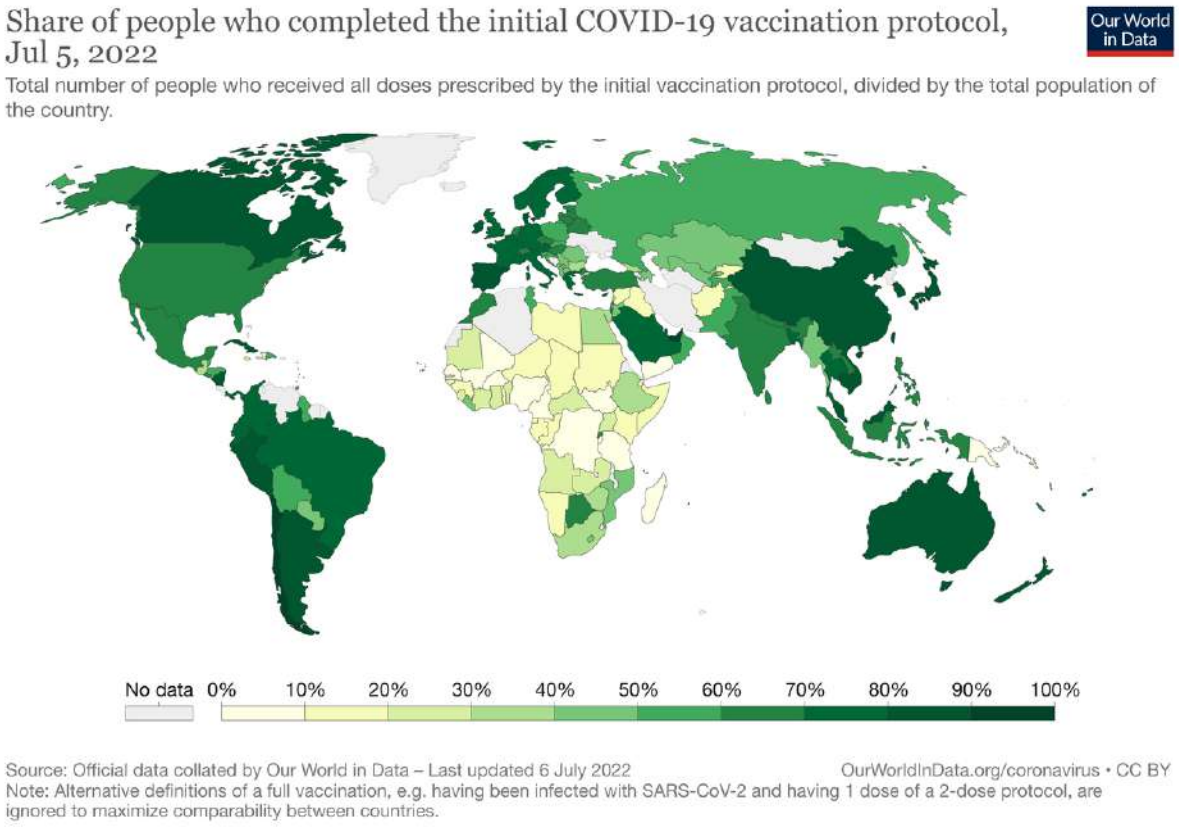

Figure 8.3: **Map showing share of population fully vaccinated against COVID-19 relative to a country's total population.**

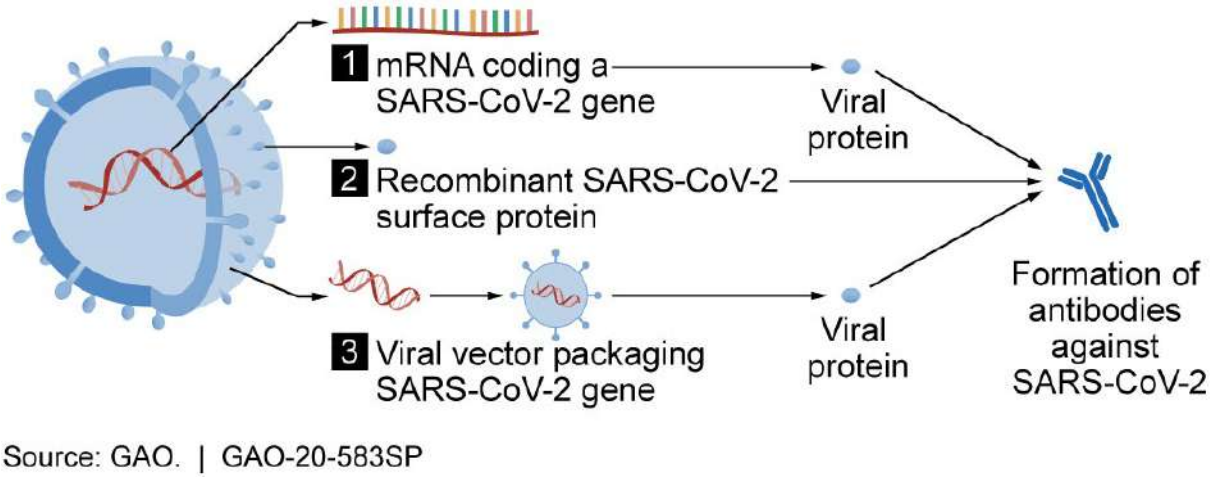

Figure 8.4: **Conceptual diagram showing three vaccine types for forming SARS-CoV-2 proteins to prompt an immune response: (1) RNA vaccine, (2) subunit vaccine, (3) viral vector vaccine.**

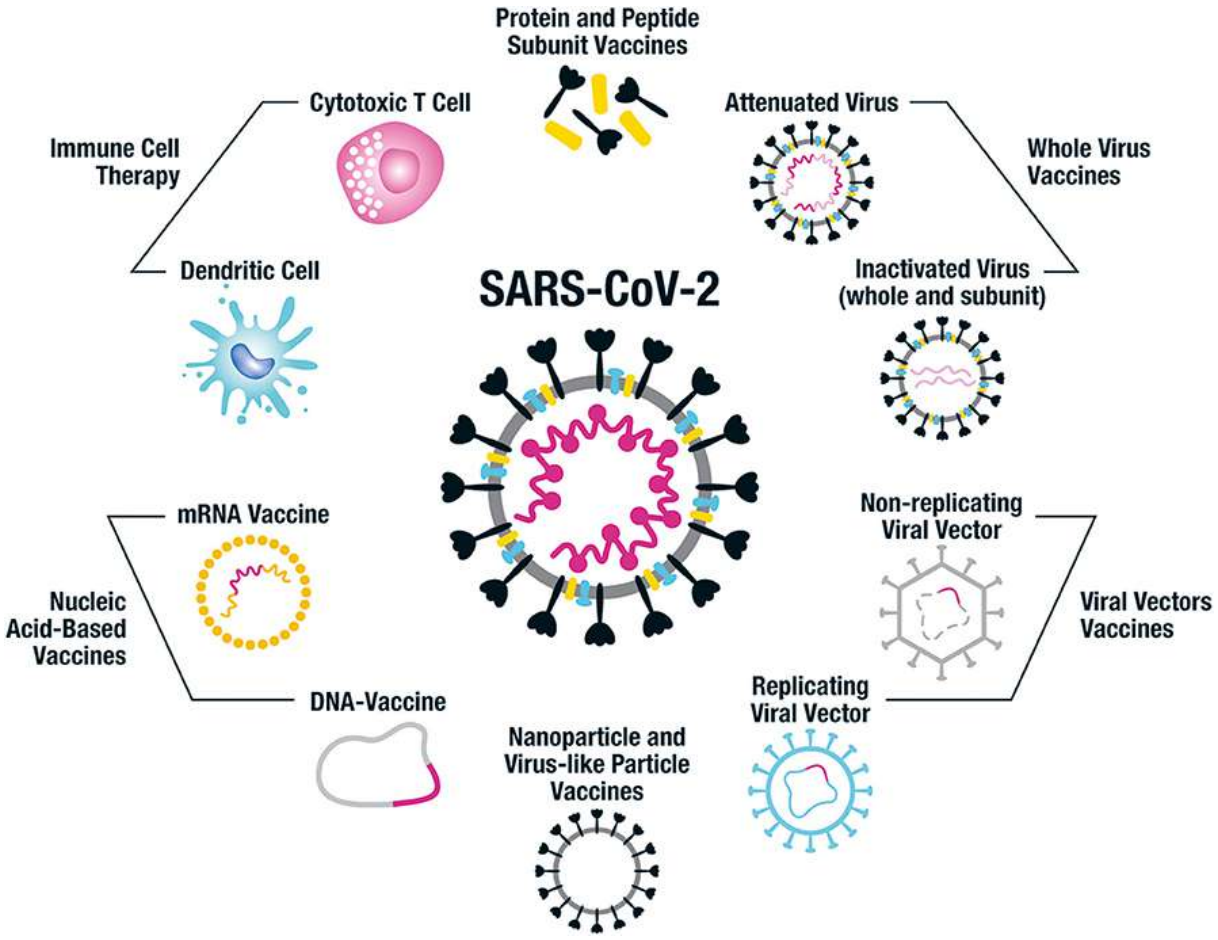

Figure 8.5: **Vaccine platforms being employed for SARS-CoV-2. Whole virus vaccines include both attenuated and inactivated forms of the virus. Protein and peptide subunit vaccines are usually combined with an adjuvant in order to enhance immunogenicity. The main emphasis in SARS-CoV-2 vaccine development has been on using the whole spike protein in its trimeric form, or components of it, such as the RBD region. Multiple non-replicating viral vector vaccines have been developed, particularly focused on adenovirus, while there has been less emphasis on the replicating viral vector constructs.**

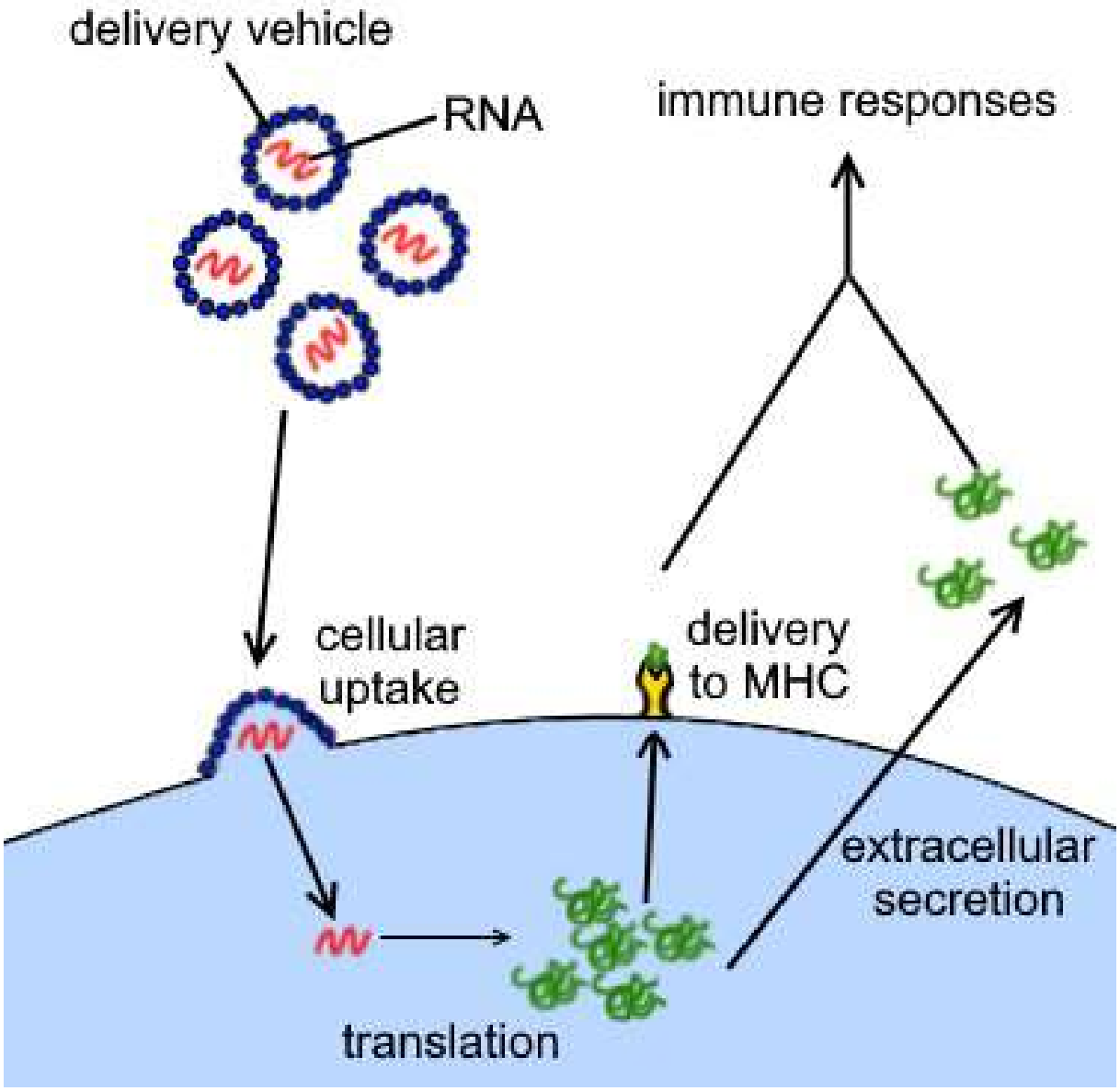

Figure 8.6: **Diagram of the operation of an RNA vaccine. Messenger RNA contained in the vaccine enters cells and is translated into foreign proteins, which trigger an immune response.**

Figure 8.7: **An elderly man receiving second dose of CoronaVac vaccine in Brazil, April 2021.**

ily members. Different countries have reacted with different rates of speed and different degrees of stringency. But the daily life of people around the world has been severely disrupted by the pandemic, and the economic consequences, already severe, will probably become worse.

A pandemic of this kind was not unexpected. Public health experts have been predicting that our world would soon be hit by a severe pandemic because air travel can take infected people almost instantly across vast distances, making local disease outbreaks global before effective limiting action can be taken.

Vaccines against COVID-19

All over the world, pharmaceutical companies committed resources to the production of vaccines for the prevention and mitigation of COVID-19, using techniques based on molecular biology. The success of this effort may be judged from a June 2022 study which estimated that "COVID-19 vaccines prevented an additional 14.4 to 19.8 million deaths in 185 countries and territories from 8 December 2020 to 8 December 2021".

Figure 8.8: **Covid vaccination for children aged 12-14 in Bhopal, India.**

Figure 8.9: **A drive-through COVID-19 vaccination center in Iran, August 2021.**

Table 8.1: **Confirmed cases and deaths as of 3 August, 2022**

Country	**cases**	**deaths**
United States	93,319,702	1,055,975
India	44,050,009	526,430
France	33,921,343	152,280
Brazil	33,890,428	679,063
Germany	31,044,554	144,360
United Kingdom	23,304,479	183,953
Italy	21,124,644	172,397
World	584,402,152	6,424,032

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