Chapter 1
Introduction: Life Is Made of Molecules!

Studying molecules is the key to understand life. A commonly accepted definition of life, known as the NASA (North American Space Agency) hypothesis, states that “Life is a self-sustained chemical system capable of undergoing Darwinian evolution” (Fig. 1.1). The link between molecules and life may be hard to explain but it is simple to illustrate.

In this introduction, we have selected three examples that are enough to show that knowledge on molecules is essential to reason about life itself, health, and disease:

1. Searching for the origin of life is a chemical “adventure” through the molecules of primitive Earth and their reactivity.
2. Viruses are amazing molecular machines, too simple to be considered living beings for most researchers, but with a tremendous ability to interfere with the course of life, sometimes tragically.
3. The world of drug discovery and development consists of molecules being designed, synthesized, and interacting with other molecules in silico, in vitro, and in vivo with the end goal of interfering with vital physiologic processes.

It is all about molecules. It is all about life.
1.1 Selected Illustrative Example #1: The Molecular Origin of Life

Nothing better than trying to answer the question “what was the origin of life?” to realize that molecules are the key to life. Since the pioneering work of Aleksandr Oparin, the origin and evolution of life are decoded based on the chemistry of molecules containing carbon. By introducing this concept, Oparin truly revolutionized the way science interprets life. Nowadays, there are two main hypotheses to explain the evolution of the complexity of molecular organization into what one today calls cells, the so-called “replicator” and “metabolism” hypotheses (Fig. 1.2). These hypotheses are based on two specific characteristics common to all living beings: despite tremendous diversity among species, all life forms are organized in cells, and all cells have a replicator polymer (DNA) and a membrane with restricted permeability (a “membrane” having lipids in its composition). Therefore, it is not surprising that the prevailing hypotheses to explain the origin of life are indeed models that elaborate on the appearance of the replicator polymer and the compartmentalization. The replicator polymer is essential to transmit the molecular inheritance from generation to generation, and a mem-
brane forming a compartment that separates the ancestral cell from its environment is essential to ensure that the molecules in this space can react among each other in a controlled and self-regulated way (a “proto-metabolism”), with minimal impact of fluctuations in environmental conditions. These two aspects are consensual among researchers who study the origin of life, but the chronological order of events that resulted in cells as we know today is far from being established.

1.1.1 The Replicator Hypothesis

According to the replicator hypothesis, life started with a molecule that was randomly formed but had the ability to replicate itself. This is an extremely unlikely event, hardly possible to occur twice in the universe, but one may work on the hypothesis that it has occurred. The obvious first “choice” for a replicator molecule is DNA, the ubiquitous replicator nowadays, but this leaves us in a paradox: proteins are needed to generate DNA and DNA is needed to generate proteins. What came first then? It may be that DNA had an ancestor with self-catalytic activity. RNA is eligible as such ancestor. RNA is not as chemically stable as DNA, so it is not so well suited to store information for long periods of time, but it can still constitute genetic material (many viruses, such as HIV, SARS-CoV-2, or dengue virus, have RNA genomes). Concomitantly, its conformation dynamics enables catalytic activity. A perfect combination for the original replicator. The introduction of mutations and other errors in replication led to evolution and selection. How this process was coupled to the appearance of a metabolism is hard to conceive, but confinement of replicators into separated environments may have favored some chemical reactions that evolved in their restrained space to form metabolism (Fig. 1.2).

1.1.2 The Metabolism Hypothesis

An alternative model skips the Achilles heel of the replicator hypothesis. Here, the origin of life is not dependent on a starting event that is nearly impossible. The key process was the confinement of small molecules that reacted among themselves. In some cases, organized ensembles of molecules may have formed stable reaction cycles that became increasingly complex. The result was the creation of metabolism and complex polymer molecules, including replicators (Fig. 1.2). Naturally, the boundaries of the confined environment where these reactions took place had to allow for selective permeation of matter. Permeation allowed growth and replication.
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Fig. 1.2 Schematic representation of the replicator (left, route 2-6) and metabolism (right, route 7-10) hypotheses to describe the origin of life. Both models are molecular in nature and agree on the critical roles of a replicator molecule and compartmentalization but differ on the sequence of events. (Figure reproduced with permission from Shapiro, Investigacion y Ciencia 371, 2007)
Nowadays, virtually all cell membranes are formed non-exclusively but mostly of lipids. Lipids are synthetic products of metabolism. So, what could have been the predecessors of lipid membranes in the confinement of the first “proto-metabolic” reactions? Cavities in the outer layers of rocks are a possibility. Phospholipids or other surfactant molecules may have started as coatings that, for their intrinsic dynamics and capability to expand and seal, may have evolved into membranes. Lipids and other surfactants can form three-dimensional structures other than lamellae that may have contributed to confine chemical systems (Fig. 1.3).

**Fig. 1.3** Types of three-dimensional assemblies of lipids. The structure of lipid assemblies depends on the degree of hydration and the molecular structure of lipids. Lipids may organize in different ways: rigid bilayers ($L_{\alpha}$), fluid bilayers ($L_{\beta}$), micelles (M), or hexagonal (H) phases.
The metabolism evolved toward self-regulation creating homeostasis, a situation in which a balance exists and small to moderate perturbations trigger responses that tend to reestablish the original, equilibrated balance. The ability of certain metabolites (intermediate molecules in a complex sequence of reactions in a living system) to activate or inhibit specific reactions in the metabolism was a major contribution to homeostasis (Fig. 1.4).

**Fig. 1.4** The evolution of networks of chemical reactions. Simple cycles of reactions (left) may have evolved in complexity (right). The interference of certain metabolites on the course of reactions possibly resulted in self-regulated metabolisms. (Figure reproduced with permission from Shapiro, Investigacion y Ciencia 371, 2007)

Nowadays, even the simplest cells, such as mycoplasma bacteria, are extremely complex systems from the chemical/molecular point of view. Considering natural evolution, all metabolisms in all living cells are related by historical bonds, and the main metabolic sequences in living beings can be drawn as “metabolic maps” (Fig. 1.5). To understand how these complex mechanisms work and do not conflict with each other, one must bear in mind that not all reactions depicted in Fig. 1.5 operate in the same species; the ones that do may not be present in all cells; in case they do, they may not operate in the same cell compartment; in case they do, they may not be working at the same time. “Complex” does not mean “confusing.” But the complexity of the metabolism as a whole is usually so high that in practice one tends to refer to “metabolisms” to designate the sectorial metabolisms in short. The word may be misleading because it may leave the impression that there are several independent metabolisms. Metabolisms are not independent of each other, and they are highly correlated, even those occurring in different organs. The need for metabolic regulation extends to the whole human body.
Fig. 1.5 A metabolic map showing a hypothetical cell, in which the whole metabolism would gather many different sectorial metabolisms: amino acids, phospholipids, steroids, lipids, saccharides, etc. (note that not all cells perform all sectorial metabolisms). (Figure reproduced with permission from International Union of Biochemistry and Molecular Biology (IUBMB))
Because metabolic pathways (sets of metabolic reactions) have evolved from the same historical background, all molecules in all living cells are also related by historical links. Their common roots determined that, despite all the apparent molecular diversity, nearly all molecules in all cells can be grouped in few families. It is also intriguing at first glance that with so many chemical elements known to man (Fig. 1.6), cells rely heavily on very few of them: hydrogen, oxygen, carbon, and nitrogen are 99% of the atoms that make a cell. How can this apparent nonsense be explained? It all resorts to the common ancestor of all living cells in all living world: these were the most abundant elements in solution in the primitive ocean. These were the founding resources and life evolved from them.

![Periodic chart of the elements](image)

**Fig. 1.6** Periodic chart of the elements. In living beings, very few elements are needed to “build” almost the totality of cells (highlighted in red), and some elements are only present in trace amounts (highlighted in pink). Yet, the elements that are rare may be absolutely essential to life. Cobalt (Co), for instance, is part of vitamin B12 (see Box 3.8)

We shall revisit in a more detailed manner the chemical nature of cells in Chap. 3.

### 1.2 Selected Illustrative Example #2: Viruses—Molecular Machines Interfering with Life

Viruses are not considered by most researchers as living beings. They are on the edge of life, able to interfere with homeostasis. They have similar molecular constituents compared to cells (proteins, lipids, nucleic acids, etc.), but there are important differences. Above all, viruses lack a metabolism of their own. Their simplicity
is not a consequence of ancestry nor does it relate to any surviving form of primitive life. Instead, might be a consequence of parasitism and regressive evolution. Alternatively, viruses may have been part of the cells. Minimal genome sizes imply faster reproduction rates for viruses and are therefore an evolutionary advantage. One may argue that viruses lack a metabolism of their own but are physical entities that are able to self-replicate and evolve, thus living beings. Even so, it is questionable whether they may be considered living because they do not replicate or evolve independently of cells. Virtually, all parasites need a host to survive and multiply, but viruses are also not able to evolve independently: they are dependent on cells to evolve because they do not have a complete own machinery of molecular synthesis.

Viruses–cell interactions are mostly physical in the early stages of the cellular infection as no chemical reactions are involved (no new covalent bonds are created nor destroyed). Let’s consider as an example the influenza virus, the virus that causes flu (there are three types of influenza viruses, A, B, and C, being the influenza A virus the major cause of seasonal flu). influenza A virus is an enveloped virus, whose genome consists of eight single-stranded RNAs that encode 11 or 12 proteins (Fig. 1.7). The virus has the protein hemagglutinin A (HA) on its surface. This protein mediates virus entry into the host cells by binding to a saccharide, the sialic acid, linked to molecules (glycans) present on the cell surface, which act as the virus receptor. HA recognizes sialic acid due to a very precise combination of hydrogen bonds and ionic interactions, among others, between well-defined atoms on the protein and on the sialic acid molecule (Fig. 1.7). These atoms, both the ones in the protein and in the saccharide, are at precise distances and orientations relative to each other so that a unique combination of forces creates a strong binding between them. Influenza A virus may establish contact with many cells in the human body but will only bind to those having sialic acid-containing receptor on its surface (mainly cells of the upper respiratory tract epithelium). Consequently, these are the cells that can be preferentially infected by the virus.

Virus–cell attachment (more precisely HA–sialic acid binding) induces virus internalization through endocytosis. The virus is enclosed in a vesicle in the cytosolic space. Upon acidification of the endocytic vesicle medium, HA is cleaved and undergoes conformational changes that result in the exposure of a terminal hydrophobic segment, called fusion peptide, which binds to the endocytic vesicle membrane. Entropy balance then serves as a driving force (see Sect. 3.1) that makes the fusion peptide to insert into the endocytic vesicle membrane. Afterward, additional changes in the conformation of the protein will bring the viral envelope and the vesicle membrane together. They are both lipid bilayers, so they collapse. Ultimately, they fuse completely and the viral content is no longer separated from the cytoplasm. The viral RNA molecules proceed to the nucleus, where they are transcribed and replicated. The transcribed viral mRNAs are translated using the cellular protein synthesis machinery. The newly synthesized viral genome is packed by some of the viral proteins forming the nucleocapsid, whereas the viral surface proteins migrate to the cell surface through the cellular secretory pathway. The nucleocapsid then associates to the surface proteins at the plasma membrane, and new viruses bud from the cells ready to initiate another infection cycle.
When two different influenza virus strains infect the same cell, the RNA of both may coexist in the nucleus. Scrambling of RNAs originates new virus having random mixtures of the genetic material of both strains. The combined viruses are usually not functional, but occasionally a new strain of increased potency may be formed. For instance, it is possible that strains of avian or porcine flu combine with human flu to form new human flu strains. These events, combined with random mutations in viral proteins, may result in extremely lethal viruses. This was the case in 1918, when a flu strain, mistakenly named “Spanish flu,” killed millions of people around the world (see Box 1.1). A mutation in a single amino acid in the HA binding site to receptors in an avian virus was enough to make it able to infect human tissues (Fig. 1.8). A small change in a molecule with a tragic impact on mankind.

Fig. 1.7  Role of hemagglutinin A–sialic acid interaction during influenza virus infection cycle. (a) Main events of influenza virus entry into a host cell. Virus binding to a sialic acid-containing receptor on cell surface is the first step of the entry process. The orientation, chemical nature, and distance of the binding amino acids of hemagglutinin A (HA) are such that sialic acid is able to engage in hydrogen bonds and other attractive forces. Upon acidification of endocytic vesicles inside the cell, HA undergoes conformational changes that bring viral and cellular membranes in contact, leading to collapse of the membranes (named fusion) from which the viral content is released inside the cell. (b) Zooming of segments of HA protein backbone contacting the sialic acid (protein carbon backbone in green; sialic acid carbon backbone in yellow)
Between April 1918 and February 1919, the world suffered the worst pandemic of modern times. Probably, it was the worst pandemics since the Black Death plague in the fourteenth century. Influenza, the virus causative of flu, infected hundreds of million people and killed, directly or indirectly, 50,000,000–100,000,000, figures so high that are hard to estimate. Europe was also being devastated by World War I. The mobility of the armies and the precarious medical assistance conditions helped spread the disease. Moreover, the horrors of war and the censorship of the news from the fronts distracted the attention of mankind to the real dimension of the pandemic, which still remains largely ignored.

Despite the common name “Spanish flu,” the disease did not start in Spain. Having a less tight censorship on the news because of its neutrality, Spain became a privileged source of information about the disease, which may have led to the impression that the disease was somehow related to Spain. In reality, the pandemic is believed to have started in the Kansas State region, in the USA, in March 1918. The new virus strain caused sudden effects, killing in a few days. In the worst cases, the patients suffered headaches, pain all over, fever, cyanosis, cough with blood, and nasal bleeding. Most deaths were associated with pneumonia, which was a consequence of opportunistic infection of lungs by bacteria. The histological properties of lungs were transformed, and there was accumulation of fluids that literally suffocated the victims, like a drowning.
Box 1.1 (continued)

The electron microscope was invented in the 1940s. Before this technical breakthrough, it was very difficult to study viruses. Other breakthroughs have followed, such as the development of super-resolution optical microscopes and PCR (polymerase chain reaction) technique, but the molecular singularities of the 1918 virus were still a challenge. The quest for the sequence of amino acids of the proteins of the 1918 strain is a story of persistence and devotion. In 1940, Johan Hultin, a medical student, spent the summer in Alaska. He heard about Teller Mission, a small missionary settlement that literally disappeared in November 1918. Seventy-two victims of flu were buried in a common grave. Later, Hultin matured the idea of recovering the 1918 flu virus from the bodies of the Teller Mission victims, presumably conserved in the Alaska permafrost. In the summer of 1951, he joined efforts with two colleagues from Iowa University, a virologist and a pathologist, and returned to Alaska to visit the former Teller Mission, meanwhile renamed Brevig Mission. With previous consent from the local tribe, Hultin obtained samples from lung tissue of some of the 1918 victims. The team tried to isolate and cultivate the virus using the most advanced techniques available but they did not succeed. It was an extreme disappointment. Hultin quit his PhD studies and specialized in pathology. Forty-six years later, in 1997, he was retired in San Francisco (California, USA) and read a scientific paper on a study of the genes of the 1918 flu strain obtained from 1918 to 1919 autopsies using PCR. Enthusiastically, Hultin resumed the intention of studying the samples from Teller/Brevig Mission. One of Hultin’s colleagues from Iowa had kept the samples since 1951 until 1996! The samples had been disposed the year before! Hultin did not quit and asked permission to repeat the 1951 sample collection. This time he found the body of an obese young woman, whose lungs had been protected by the low temperatures and the layer of fat around them. The complete genome from the 1918 flu strain was obtained from these samples.

The hemagglutinin sequence of the 1918 strain (H1) was reconstructed from the genome of the virus. The sialic acid binding site underwent mutations in the amino acid residues relative to avian flu (H5) that enlarged the binding site, enabling the mutated viruses to bind and infect human cells. The modern studies on the phylogenetic tree of the flu viruses, which now include data from samples from South Carolina, New York, and Brevig, all from 1918, relate the origin of the virus to an avian strain found in a goose (Alaska 1917) (see below figure). Although this hypothesis is not totally consensual among researchers, the fear that new unusually deadly flu strains adapted to humans evolve from avian flu strains persists and is a matter of thorough surveillance of health authorities around the world. In 2020, another respiratory virus, SARS-CoV-2, causative of the COVID-19, crossed the inter-species barrier, presumably from bats to humans, and a world wide pandemic started.

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1.3 Selected Illustrative Example #3: Molecules as Tools—Drug Discovery and Development

Designing new drugs that can be developed into new medicines demands knowledge on the role of different molecules in different pathologies. A molecular-level target is needed for the drug candidate, and the researcher needs to have an idea on how they are going to interact so that the target can be inhibited or activated. A drug candidate that is targeted to a protein, such as an enzyme or a membrane receptor, for instance, needs a binding site where it can react or attach both strongly and selectively. “Selectively” means it will discriminate this site from all others in the Phylogenetic tree of the flu viruses. (Figure reproduced with permission from Taubenberger et al., Scientific American 292, 2009)

Box 1.1 (continued)
same target or in any other molecule of the body. The uniqueness of the binding site is granted by the precise arrangement of the atoms in space. Ideally, only that site has the right atoms at the right distance, in the right orientation to maximize intermolecular attraction forces (see the example of HA–sialic acid binding in Fig. 1.8). Hydrogen bonding, ionic/electrostatic forces, van der Waals interactions, etc., all these are dependent on the spatial arrangement of elements of both drug candidate and target. The Beckett–Casy model for opioid receptors illustrates the basics of these principles (Fig. 1.9). In addition to the efficacy in binding to its target, drug molecules need not to be exceedingly toxic or have significant other undesired effects, which is directly related to its reactivity and selectivity.

**Fig. 1.9** Beckett–Casy hypothesis for the binding of an analgesic molecule (such as morphine, which is illustrated) to an opioid receptor. While the exact structure of the receptor is not known, the key interaction/attraction forces were identified: electrostatic attraction, H bonds, and van der Waals interactions. The receptor is so specific for the ligand that chiral molecules (bearing the same chemical groups but with different orientation in space) do not fit

The same reasoning applies to complex therapeutic molecules such as antibodies. Let us take as an example one of the antibodies that targets the protein gp120 on the surface of the HIV (Fig. 1.10). This protein is responsible for binding to the receptors and co-receptors for the virus on the T-lymphocytes, this being the first step of infection. When an antibody binds to gp120, it may block the access of gp120 to the receptors and/or co-receptors, thus preventing infection. Anti-HIV antibodies are hopes for future therapeutics although the rate of mutations in gp120 and the presence of glycosylated groups on gp120 surface pose problems that are difficult to overcome.
Some researchers devote their work to antibody engineering, i.e., the manipulation of antibodies for a specific purpose. Some try to find the smallest portion of an antibody that is still active, so that antibody therapy can be made simpler and more cost-effective. Manipulating antibodies demands knowledge on the molecular-level interactions they perform with their antigens. At this level, the forces that are responsible for selectivity and strength of binding are not different from those that small molecules (such as the opioids in Fig. 1.9) establish with their molecular tar-
gets, but the number of bonds (hydrogen bonding, electrostatic interactions, van der Waals interactions, etc.) involved may be higher, resulting in extreme selectivity and very strong binding.

The whole process of devising and studying drugs (frequently termed “pipeline”) has three main stages: research, development, and registration (Fig. 1.11). The research stage is typically, but not exclusively, carried out at universities and academic research centers. During this phase, relevant targets for selected pathologies are identified, and a molecule to interfere with that target is selected. This molecule is a drug candidate that can be improved. Such molecule is termed “lead” and the process of improvement is termed lead optimization. Research stage takes several years (rarely less than 5).

**Fig. 1.11** The drug discovery and development process, generally termed “pipeline” in pharmaceutical industries. There are three main stages: research, development, and registration (center), distributed over several years (numerical timeline). Each stage is divided in sub-phases. During the research stage, relevant targets for determined pathologies are identified, and molecules to interfere with those targets (“leads”) are selected. The development stage may proceed for the next 7 years, during which the drug leads are tested for safety and efficacy in carefully designed animal and human clinical trials. At the ending of each phase, the results are evaluated; safety and/or efficacy issues may prevent further tests. For each nearly 1000 molecules starting the process, only one ends the last stage successfully. This failure ratio, generally named “attrition rate,” is incredibly high. Moreover, not all molecules are granted approval to enter the market for regulatory reasons, and those that enter the market are still monitored afterward (phase IV).
The pre-clinical development is the first step in the development stage and the last step before clinical trials. Pre-clinical studies consist in as many experiments in vivo and in vitro (both in cells and artificial systems) as needed to ensure that a certain optimized lead is safe at a certain dosage range when prepared as a specific selected formulation using a defined mode of administration. The goal is to minimize risks to the lowest possible level when administering the optimized lead to humans. Efficacy comes after safety in the priority list. This is the reason why the first tests in human (phase I clinical trials) are performed in few healthy volunteers, not patients. At this phase, safety is tested using conservative doses of the compounds under study. Tolerability, absorption, and distribution in the body and excretion are followed. Phase II clinical trials include patients, and efficacy, besides safety, is also tested. The drugs are administered to up to several hundred individuals for several weeks or a few months, typically. The dose range of the drug is improved during the trials. It should be stressed that all trials are scientifically controlled for the statistical significance of the results. The placebo effect (Box 1.2) is also accounted for in the trials. The process of designing clinical trials, data collection, and meaningful data analysis for reliable conclusion is, by itself, a complex discipline.

**Box 1.2 The Placebo Effect: The Power of Nothing**
*(Based on “The power of nothing” by Michael Specter in The New Yorker December 12, 2011, Issue)*

A placebo is a simulated or otherwise medically ineffectual treatment for a disease or other medical condition intended to deceive the recipient. Sometimes patients given a placebo treatment will have a perceived or actual improvement in a medical condition, a phenomenon commonly called the placebo effect.

For most of human history, placebos were a fundamental tool in any physician’s armamentarium—sometimes the only tool. When there was nothing else to offer, placebos were a salve. The word itself comes from the Latin for “I will please.” In medieval times, hired mourners participating in Vespers for the Dead often chanted the ninth line of Psalm 116: “I shall please the dead in the land of the living.” Because the mourners were hired, their emotions were considered insincere. People called them “placebos.” The word has always carried mixed connotations. Placebos are often regarded as a “pious fraud” because bread pills, drops of colored water, and powders of hickory ashes, for instance, may sometimes lead to a perceived improvement in patients.

The first publicly acknowledged placebo-controlled trial—and still among the most remarkable—took place at the request of King Louis XVI, in 1784. The German physician Franz Anton Mesmer had become famous in Vienna for a new treatment he called “animal magnetism,” and he claimed to have discovered a healing fluid that could “cure” many ailments. Mesmer became highly

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Phase III clinical trials are a replica of the phase II trials, but several thousands of individuals are enrolled and treatment may be extended in time. Phase III is thus a refinement of phase II both in terms of efficacy and safety. Rare events such as unlikely undesired off-target effects that may have not been detected in phase II are now more likely to be detected. Concern for rare undesired off-target effects that may jeopardize the safety of drugs, even to small and very specific subpopulations of patients, is always present, even after the drug has been approved as a medicine for clinical use. This is sometimes termed “phase IV” and consists in screening how the drugs perform in the “real world,” outside a tightly controlled environment.
Regulatory approval follows phase III and precedes phase IV and initiates the registration stage. The results of the development of the drug, from molecule to man, from bench to bedside, are submitted to regulatory agencies, which assess the results and conclusions of the whole clinical development based on the evaluation carried out by independent experts. The need for that specific new drug and how innovative it is when compared with existing drugs for the same purpose are also taken into consideration. The decision on allowing a specific molecule to be part of a new medicine or not belongs to these agencies.

The numbers associated with the difficulty in developing a successful drug, which is later incorporated in a new medicine, are absolutely impressive. For each 1–5 million “new chemical entities” (molecules tested for their pharmacological interest), only 1000 have positive results in in vitro tests, from which only 70 are selected as leads, which are then optimized to form seven drug candidates that enter clinical trials. Out of these seven, only 2–3 reach phase III clinical trials and only one is approved by regulatory entities. The whole process takes around 15 years to complete (Fig. 1.11) and has an estimated total cost of several millions of USD for each approved new drug, on average. It is important to point out that these are very crude numbers that vary a lot for different areas of medicine, but they serve to illustrate the efforts needed to continuously fight against disease progress. Reducing the attrition rate (fraction of new chemical entities that fail during the drug development process), accelerating the whole process, and making it more cost-effective are hugely demanding but urgently needed tasks.

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Challenging Case 1.1: The Origin of Life and Panspermia

Source

This case is based on the classic novel by H.G. Wells, *The War of the Worlds,*\(^1\) that although written more than a century ago, remains contemporary, having been adapted many times to different media, from a 1938 radio play to a Hollywood film directed by Steven Spielberg in 2005. It is also a source of inspiration for other productions. It brilliantly highlights the collective wishful thinking expressed by science fiction writers that not only are we not alone in the universe but also that life’s origin may follow similar trajectories, a point that is still hotly debated today.

Case Description

In real life, humans do not seem to display much love or respect for their planet as judged by the overwhelming neglect that has led to widespread degradation of air, land, and sea. This unfortunate human trait is not justifiable in terms of ignorance—everyone knows what’s happening—and yet the visible global damage now approaching the point of no return does not scare anyone into action. Curiously, as far as fiction goes, all it takes to provoke an about-turn is a threat, or actual invasion by aliens. Then, humans join forces and zealously guard the Earth against all odds and sophisticated weaponry. Although the record shows numerous tales of attempted alien invasion, perhaps *The War of the Worlds* translates faithfully our angst regarding the unknown.

Although *The War of the Worlds* was written in 1898, it still holds its own against contemporary productions, such as Carl Sagan’s “Contact” (1985); “Independence Day” (1996), by Roland Emmerich; and “Arrival” (2016), by Denis Villeneuve. Briefly, *The War of the Worlds* begins when British astronomers detect strange explosions on the surface of Mars. This is followed by the arrival of a meteorite in Woking, Surrey. The narrator then witnesses the emergence of the Martians from artificial cylinders. They were described as big and grayish and with oily brown skin. The Martians did not agree with the local atmosphere and Earth’s gravity, and so they retreat back into the cylinders from where they go on masterminding the

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\(^1\) Wells HG (1898) The war of the worlds, 1st edn. Harper & Bros (US) Publishers.; 287 p, Heinemann.
invasion. From the start, they reject human attempts to establish contact by promptly dispatching the ambassadors to be with powerful heat rays. After the peace talks fail, war begins and there is much destruction and pain. The Martian heat rays and a black smoke go on producing havoc, and gradually it dawns on the main characters that along with destruction the Martians are also feeding on the humans that get caught by their tripod fighting machines. They also try some mars forming (as opposed to terraforming) by spreading some weed that grows luxuriantly wherever there is abundant water available. The humans are taking a beating, but suddenly when everything seems to be lost and the Earth is finally going to be relinquished, the Martians are found dead. Every one of them. It turns out they were killed by earthly pathogens against which they had no immunity. And so the story ends.

*The War of the Worlds* has been amply discussed by scholars and many interpretations were put forward. Surely, having witnessed wars in Europe, Wells had many reasons to be ambivalent about technology, as well as pessimistic about the human’s ability to cope with the environment. Thomas Henry Huxley, Darwin’s bulldog, also asserted that *The War of the Worlds* was an example of the struggle for survival. Wells himself betrays his xenophobic views vis-à-vis his description of the Martians. The list goes on. On the other hand, as a biologist, Wells must have given some thought to the question of the origin of life when writing *The War of the Worlds*. This is clearly expressed as the plot unravels.

In *The War of the Worlds*, human armies are inoffensive, powerless to stop the advance of Martians over our territory. Yet, the powerful aliens soon fall dead from the contagion by bacteria. Our ancestor enemies were merciless: Martians had no resistance to Earth bacteria. The importance and need for antibiotics are certainly felt differently after listening to the adaptation of the visionary novel of H.G. Wells to radio or Spielberg’s movie.

**Questions**

1. Select from the text above a passage that indicates that the appearance of life in the universe depends on the same scenario as that on Earth.
2. What does the fact that the Martians were feeding on humans tell about the physiology of the aliens?
3. After less than 80 years from the beginning of the mass use of antibiotics, these miracle drugs are failing. Resistant infections kill hundreds of thousands of people around the world each year, and there are now dozens of so-called superbugs.
   (a) Relate the excessive use of antibiotics with the emergence of bacterial resistance.
   (b) Is excessive use a consequence of excessive prescription by doctors?
Biochemical Insights

1. H.G. Wells’ text mentions that the Martian weed had a very strict requirement for water, in the same way as the terrestrial plants, or for that matter, all other life forms on Earth. Indeed, whenever astrobiologists—scientists seeking life in the universe (see more about astrobiology in the Challenging Case 1.3)—investigate a planet in our solar system or outside (exoplanets), the first question they ask is: “Is there water there?” Why water? Water has many physical–chemical properties that according to our knowledge of life and its origins make it a very versatile solvent (see Chap. 2). For example, water is a dipole (the same molecule has two opposite charges), that is, water molecules are able to surround various types of polar solutes and, in doing so, dissolve them. This is only possible because the interactions between water and several solutes are energetically more favorable than the interactions between water molecules. Also, the polar nature of water makes it immiscible with hydrophobic compounds such as lipids. This kind of interaction favors the spontaneous formation of membranes and also helps to organize the structures typical of different intracellular cellular organelles. In other words, water is able to organize structures in which hydrophobic compounds prevail. Water is also a reagent that participates in many biochemical reactions of the living cell, such as enzymatic reactions. And so on and so forth. In contrast, other types of solvents are more limited in terms of their physical–chemical properties. In addition, water is ubiquitous in the universe. It is thought that in our solar system, some planets and their moons contain frozen water or liquid water underground. So, it is reasonable to propose that, if conditions were right, life in other planets could have a similar origin as far as water is concerned. Naturally, other building blocks would have to be involved (see below). Similar arguments could be put forward for the element carbon. We know for a fact that life, as we know it, is based on the chemistry of carbon. Why carbon? Why was this element selected as a major constituent of living beings? Certainly, that was not a capricious choice. Carbon is able to form bonds with itself and with many different elements, such as nitrogen, oxygen, and sulfur. These in turn can form many radicals that, by reacting with each other, can form various compounds. The ability to form bonds that are at the same time not too stable or unstable also counts favorably within the context of life. In general, stable compounds tend to be unreactive, and very unstable compounds do not remain intact for a significant length of time. Another relevant property of carbon is associated with rotation of bonds that permit the formation of tri-dimensional structures that add to the variety of carbon compounds. Rigid structures, on the other hand, limit the way in which compounds can interact with other molecules. In addition to reactivity, there seems to be no limit to the size and variety of carbon compounds. It is estimated that today approximately nine million carbon compounds exist in nature. So, it is not surprising that among all these possibilities, some actually did contribute to establish the intricate networks of interacting compounds that are the basis of the biochemical reactions (chemical reactions of the living cell).
Carbon is also abundant in the universe. It is formed in the stars and is one of the main constituents of nebulae. Therefore, an exoplanet that has water and carbon could in principle contain the main ingredients that eventually would give origin to life. Even though we could consider the elements to be universal, this does not mean that living beings on other planets besides Earth would be exactly the same as the ones we observe here. They may have similar biochemistry, however. As we know, the diversity of living beings is conditioned by evolution. By examining the fossils of animals that existed millions of years ago, we can appreciate that those living forms are as different from many extant ones as alien life would be from the terrestrial ones.

2. The feeding habits of the Martian invaders are similar to the earthlings. Despite their exotic properties and advanced technology, they too cannot escape from the universal dependency on energy. Hence, the Martians had to harness energy through the ingestion of other living beings just as we do on Earth. Besides, just like us, depending on what we eat, there might be nefarious consequences. If one ingests contaminated food, containing either bacteria or parasites, we may become sick. Sometimes fatally, like the Martians in the story. Their organisms did not agree with some of the components of humans, presumably the billions of bacteria that live in our gut and that normally are harmless to us. Again, H.G. Wells’ description of the aliens allow us to perceive that he was a true evolutionist and as such believed that depending on the environment and the chemistry that would occur with the existing elements, life elsewhere could generate a myriad of creatures.

3. Misuse of antibiotics spreads across many different private and professional activities and cannot be assigned exclusively to excessive prescription. “The increasing global availability of these drugs over time since the 1950s, their uncontrolled sale resulting in broad-spectrum antibiotics being prescribed when not indicated, as well as antibiotic use in livestock feed at low doses for growth promotion and the releasing of large quantities of these medicines into the environment during pharmaceutical manufacturing”² are the main causes of excessive environmental presence of antibiotics in a broad sense. This has driven a massive process of killing of antibiotic-sensitive bacteria. Concomitantly, antibiotic-resistant bacteria thrive and multiply, colonizing areas that would naturally be occupied by antibiotic-sensitive bacteria. The ubiquitous presence of antibiotics has thus led to a fast and prosperous selection process for antibiotic-resistant bacteria.

²Riva E (2015) The war of the worlds and antibiotic resistance: a case study for science teaching. Biochemist 37:26–28.
Final Discussion

Although we don’t really know how life appeared on Earth, there are several hypotheses based exclusively on physical–chemical explanations, as addressed in section 1.1. The general idea is that, during the prebiotic era (before life), small molecules such as amino acids, monosaccharides, and purine and pyrimidine bases were synthesized from existing elements (carbon, nitrogen, oxygen, hydrogen) using thermal, electric, or UV energy. This has been experimentally confirmed. In time, small molecules reacted with each other and eventually generated polymers. It is also known that membranes and, therefore, compartments can form spontaneously when amphipathic hydrocarbons are exposed to aqueous solutions. Compartment formation as such was an important step because by increasing the local concentrations of the reagents, several reactions would be favored. The next stages would comprise the organization of the molecules into coherent pathways that were the precursors of metabolism.

The “appearance” of life could thus be regarded as resulting from the gradual increase in complexity that ultimately manifested itself by displaying the recognized features of a living cell. As life evolved on Earth, multicellular organisms were formed, lived for a while, and went extinct. This brief description of the events from prebiotic chemistry to living beings sums up the scientific thought, which is broadly consensual among scientists, giving or taking this or that pathway. By accepting the materialistic view on the origin of life on Earth, it is plausible to generalize it to the rest of the universe. When astrobiologists ponder about extraterrestrial life, they invariably question whether the planet in question lies within the comfort zone determined by its location in relation to that of the nearest star (the planet can’t be too cold or too hot), whether it has water and an atmosphere. By doing so, they agree that life elsewhere followed the same trajectory as on Earth. Challenging Case 1.3 will revisit this theme.

This case is a contribution of Prof. Franklin D. Rumjanek (Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Brazil). In memoriam (1945-2020).
In 2015, the Biochemical Society, UK, dedicated a special theme of its magazine *The Biochemist* to “Biochemistry on screen.” One of the articles, by Nick Loman and Jennifer Gardy, titled “Contagion: a worthy entrant in the outbreak film genre,” pinpoints the realistic and unrealistic aspects of the scientific setting in the fictional Steven Soderbergh’s movie “Contagion” (2011). The exploration of the overlap between science and fiction in an intense drama paved with fear creates an opportunity to address the molecular aspects of viral epidemiology.

Cover of magazine *The Biochemist* vol. 37, No. 6, December 2015

**Case Description**

The clash between powerful rulers and unprotected victims of abuse is a common theme in art, sometimes to convey alert messages for a better society, other times just to fuel an intense storyboard. Occasionally, these dramas happen in scientific scenarios, and unusual characters such as aliens or microbia star in the main roles of the script. This was certainly the case of *The War of the Worlds* (see Challenging Case 1.1), as well as the Steven Soderbergh’s film “Contagion” (2011). Both examples deserve attention as the search for the boundaries that divide science from fiction is certainly more challenging and enticing than meets the eye at first glance. H.G. Wells’ original novel is completely fictional and tells the story of a Martian invasion of Earth. “Contagion” is a film that mirrors recent scares of viral outbreaks such as severe acute respiratory syndrome (SARS), COVID-19, or Ebola. In both cases, it is evident how the discovery, development, mass production, and distribution of antibiotic and vaccines (since 1940s) revolutionized medicine and changed human societies in every single aspect from agriculture to war and … art. Importantly, it is also implied how the emergence of antibiotic resistance or anti-vaccine attitudes may revert past achievements and changes the tide in favor of microbes.

“Contagion” is focused on routine aspects of epidemiology work, as Nick Loman and Jennifer Gardy put it: “The paper notebooks and whiteboard sketches trying to link cases to each other, the frustration that comes with trying to keep a case count going across hundreds, if not thousands, of jurisdictions, and the constant querying around precisely whose budget things are to come out of. Contagion gets a great deal right. The fictional virus, MEV-1, is borrowed from the Nipah virus (a newly emerged zoonosis that causes severe disease in both animals and humans) including the bat-to-pig-to-human “spillover” event that launches the outbreak, and the SARS
coronavirus, which, like its celluloid cousin, used Hong Kong and its crowded apartment complexes and hotels as a stepping stone to global spread. The uncertainty in the early days of the outbreak is also clear, from the medical doctor who can’t offer a diagnosis to the epidemiologist trying to calculate $R_0$, the virus’ reproductive ratio to estimate the potential scale of the problem.

Uncertainty spreads in the population, causing fear, misplaced belief in sham remedies, and manipulation, a set as dangerous as viruses themselves. We have all witnessed these events in real life during recent viral outbreaks or pandemics, from influenza to Ebola, SARS-CoV-2, dengue, or Zika. On the other hand, fiction has its own limitations and even scientific scenarios must be adapted for storytelling. Realism would cost a lot of repetitive testing, incomplete models, iterative thinking, collective data sharing, and other sorts of action difficult to fit in a linear, thrilling, clear-cut narrative. Surely, a pandemic striking one in every 12 people in our planet would take more than two epidemiologists to handle the case, and buzzwords, like sequencing or receptor entry, have more precise meanings for biochemists than lay audiences can perceive. Interestingly, in sequencing, reality has overcome fiction as modern portable sequencing technologies can read all nucleic acids from pathogen and host cells present in a sample rapidly. Phylogenetic trees of outbreaks can then be used to identify epidemiological patterns, related hotspot communities, and cross-border spread, for instance. Overall, “Contagion” helps in realizing that scientific knowledge is the key to manage and ultimately eradicate viral deadly menaces. “The question when it comes to the next great pandemics is not if, but when.” In 2020, SARS-CoV-2 reminded the world of this reality.

Questions

1. $R_0$, the “basic reproduction number” of an infection, is a tool that epidemiologists use to understand an infectious disease’s potential to spread among a population. It describes the average number of people that one sick person will ultimately infect during an outbreak—a disease with an $R_0$ of 2, for example (something like SARS-CoV-2 or Ebola virus $R_0$), is one in which every one sick person will go on to infect two more people. The $R_0$ of a disease isn’t set in stone—it will vary over the course of an outbreak or epidemic and depends on characteristics of the host, pathogen, and environment—but the general rule is that when $R_0$ is less than 1, the outbreak will eventually die out.
   (a) For Ebola, calculate the progression in number of people affected as the virus spreads.
   (b) How do you translate its progression into a mathematical formulation?
   (c) Search the literature and find which virus has the highest $R_0$. Compare disease progression for this case and Ebola ($R_0 = 2$).

2. Why viral genome sequencing and knowledge on the receptors used by the viruses to entry the cells are crucial to devise strategies to develop drugs against viruses?

3 Loman N, Gardy J (2015) Contagion: a worthy entrant in the outbreak film genre. Biochemist 37:22–25.
Biochemical Insights

1. First infection: 1
   Secondary infection: 2 + 1
   Tertiary infection: 4 + 2 + 1
   … + 8 + 4 + 2 + 1

   \[ N = R_0^n + R_0^{n-1} + R_0^{n-2} + \cdots = \sum_{i=0}^{n} R_0^{n-i} \]

   \[ t = n \times \Delta t \]

   (\(N\), total number of infected people; \(n\), order of infection; \(\Delta t\), the average interval between consecutive infections)

   The highest \(R_0\) values are found for measles virus and rotavirus infections, which have \(R_0 \sim 15\) (sometimes even higher), as illustrated in the following figure:

   [Image of a scatter plot showing the correlation between \(R_0\) and deadliness for various infections.]
The number of affected people is expected to progress as depicted in the following figure. \( \Delta t \) were chosen so that the model data could come close to the experimental data of the Ebola outbreak in Liberia in 2014.\(^4\) Other outbreaks cannot be described using such a simple model and demand more sophisticated calculations using, e.g., fraction of susceptible and exposed individuals, who can either recover and survive or die, and duration of incubation and infectiousness and fatality rate.

![Infection progression for viruses with different \( R_0 \). Expected theoretical progression (solid lines) of the number of people infected with a virus with a \( R_0 = 15 \) (experimental value for measles virus) or \( R_0 = 2 \) (experimental value for Ebola) for \( \Delta t = 18 \) (orange and green lines, respectively). \( \Delta t = 18 \) was selected so the theoretical expectation could fit the data from an Ebola outbreak in Liberia in 2014\(^4\) (green circles). For the sake of comparison, \( R_0 = 2 \) for \( \Delta t = 21 \) is also represented (i.e., slower rate of transmission). The data for the outbreaks of Ebola in Guinea (yellow circles) and Sierra Leone (blue circles) are not adequately described by the simple model developed in Answer 2 and need more sophisticated models\(^5\).

2. Genome sequencing allows the determination of viral protein sequences and their posterior identification, so that viral proteins can be targeted by drugs and antibodies. Likewise, discovery of the receptors used by the viruses to enter the cells is important because they may be druggable, i.e., prone to be targeted by molecules that abrogate viral infection through receptor blocking or inactivation. Moreover, receptor identification may explain why some tissues in the body are infected and others are not.

3. Many antibacterial drugs inhibit enzymes or transporters that take part in bacterial metabolism. Viruses lack a metabolism of their own. Instead, they modulate the metabolism of the host.

4. DNA is much more stable than RNA (see Fig. 3.29). Frequent chemical alterations in RNA cause high rates of mutation in RNA viruses as compared to DNA.

\(^4\)Althaus CL (2014) Estimating the reproduction number of Ebola virus (EBOV) during the 2014 outbreak in West Africa. PLoS Curr 6.
ones. Additionally, RNA viruses use their own RNA-dependent RNA polymerases to replicate, which lack the proofreading activities present in DNA polymerases. Mutations affect the structure of proteins, which may alter the binding sites of drugs and antibodies (epitopes).

5. Places like big cities in Asia are ideal settings for new virus to emerge and propagate because street markets with live animals are abundant, population density is high, and as cosmopolitan urban communities, lots of people are entering and leaving every day. SARS started in China and spread to the world from Hong Kong; COVID-19 pandemic started in Wuhan, China. New influenza strains spread throughout the world every year starting from this area, albeit not exclusively.

6. Developing a new vaccine is very laborious and time consuming. Besides creating the vaccine prototype, testing itself demands years of thorough data retrieval and processing. On top, production is usually not trivial and is thus time demanding. In this regard, “Contagion” is not realistic.

Final Discussion

Science fiction seems to have evolved into the new era of science in fiction. Movies such “E.T., The extraterrestrial” (1982) and “The Rock” (1996), in which biochemistry is central for script, made the transition. The successes of TV series like “The Big Bang Theory” and “Breaking Bad” consolidated the awareness of the asset science setting and scientists represent to Hollywood and entertainment industry. The National Academy of Sciences of the USA created the program “The Science & Entertainment Exchange” (“The Exchange,” http://scienceandentertainmentexchange.org/), which connects entertainment industry professionals with top scientists and engineers to create a synergy between accurate science and engaging storylines in both film and TV programming. The goal of “The Exchange” is to use the vehicle of popular entertainment media to deliver sometimes subtle, but nevertheless powerful, messages about science.

The frontier where science meets art, communication, and entertainment is certainly fertile for teaching and learning biochemistry inasmuch the public is educated in science matters, so facts are distinguished from fiction and the best of both worlds can be appreciated for the benefit of all.
Challenging Case 1.3: Mars—NASA’s Laboratory for the Origin of Life ... On Earth!

Source

This case is based on real data published in the scientific literature. NASA, the National Aeronautics and Space Administration of the USA, has a Program focused on Mars. Among the aims of the Mars Exploration Program, two are very relevant to biochemistry: to determine if Mars ever supported life and to determine if chemical events taking place on Mars can be extrapolated to what is thought as being the early stages of life on Earth. The end goal of NASA is to prepare for human exploration of Mars, which also demands knowledge on human biochemistry in hypobaric conditions. Find out more at https://mars.nasa.gov/.

Case Description

The mission statement of the Mars Exploration Program is clear: “The goal (...) is to explore Mars and provide a continuous flow of scientific information and discovery through a carefully selected series of robotic orbiters, landers and mobile laboratories interconnected by a high-bandwidth Mars/Earth communications network.” In fact, the Program is a science-driven, technology-enabled study of Mars as a planetary system in order to understand, among other issues, the potential for Mars to have hosted life and how Mars compares to and contrasts with Earth in present or past times.

The quest for signs of life on Mars is the most interesting endeavor for a biochemist spectator of the Mars Exploration Program. To begin with, identification of the signs of life is not trivial as living beings are made of non-living matter. The molecular “building blocks” of life are not alive themselves. Life is a complex organization of matter, and the frontier between living and non-living is not sharp for a biochemist. So, what to look for when looking for life? To help them in the complex task of finding the answer to this question, NASA scientists created what they called the “Life Detection Ladder,” from the basic requirements essential for molecules to organize and originate life to other more sophisticated forms of very specific molecular interactions. In creating the ladder, researchers started with the NASA definition...
of life, “Life is a self-sustaining chemical system capable of Darwinian evolution,” and considered the specific features of the one life known, which is terrestrial life.\(^5\)

Water, presence of molecular building blocks for use, an energy source, and gradients (i.e., confinement of sets of molecules) are the basic essential conditions to originate life. The presence of moderately complex organic molecules deviating from background bulk concentration and having chiral centers would be a subsequent stage in the signs of life path. Functional macromolecules, metabolism, and highly adaptative systems are higher-order signs, but they are not so relevant because no signs of such kind have been detected yet in any circumstance outside Earth. In practice, the quest for signs of life so far has only been a matter of detecting water and simple organic molecules and coping with elusive hints of eventual bacteria-like encapsulation structures that may have allowed molecular confinement/compartimentalization.

Life on the surface of Mars is unlikely given the presence of very reactive oxygen species that “attack” organic molecules, degrading them. However, inside rocks and deep below the surface, using geothermal energy, life is, in principle, possible … Inasmuch there is water! This is the reason why NASA researchers are searching for evidence of liquid water that exists or has existed on Mars. In January 2018,\(^6\) NASA reported that researchers have found eight sites where thick deposits of ice beneath Mars surface are exposed in faces of eroding slopes. The ice was likely deposited as snow long ago, providing clues about climate history. The sites are both in northern and southern hemispheres of Mars, at latitudes from about 55° to 58°, equivalent on Earth to Scotland or the tip of South America. Six months later, in July 2018, Italian scientists from the European Space Agency reported indirect evidence that there is a frozen water lake in Planum Australe, at the Martian south pole, having liquid water underneath.\(^7\) This lake extends through 20 km and reaches 1.5 km deep. Liquid state is putatively assigned to high contents in Mg, Na, and Ca ions, from the rocky soil, and pressure caused by the thick ice layer on the surface.

Ten years earlier, the Phoenix mission had already detected and analyzed buried water ice at 68° north latitude. Ice was directly exposed on the surface and sublimated, from solid into water vapor. Recent studies reached similar findings.\(^8\) It is known that that most of the current Martian atmosphere consists of carbon dioxide. If water and gases in the atmosphere reacted and formed the carbonate minerals on the Martian surface by chemical reactions, the presence of these minerals is a clue that water had been present for long enough for life to have developed. If this was the case, sedimentary rocks would certainly keep traces of microscopic life forms, like protobacteria: Martian fossils! The claim that fossils of microbes had been

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\(^5\) Life detection ladder. Astrobiology newsletters. NASA. [https://astrobiology.nasa.gov/research/life-detection/ladder/](https://astrobiology.nasa.gov/research/life-detection/ladder/).

\(^6\) Dundas CM et al (2018) Exposed subsurface ice sheets in the Martian mid-latitudes. Science 359:199–201.

\(^7\) Orosei R et al (2018) Radar evidence of subglacial liquid water on Mars. Science 361:490–493.

\(^8\) Piqueux S et al (2019) Widespread shallow water ice on mars at high and mid latitudes. Geophys Res Lett 46(24).
found in Martian meteorite ALH840019 originated one of the most intense and passionate scientific controversies in modern science together with cloning of the sheep Dolly, both occurring in 1996. The hypothesis was finally considered unreasonable because it was not totally supported by evidence.10

In 1976, NASA’s two Viking landers made first the attempt to retrieve organic matter on Mars but did not succeed. In June 2018, new data was disclosed by NASA on the existence of organic matter on Mars, namely, in 3-billion-year-old mudstones in the Gale crater region.11 Three years before, in 2015, the Curiosity rover drilled into the mudstone called “Mojave,” and the analysis of the cuttings yielded organic molecules. The analyses were carried out automatically at a miniaturized automatic lab; the results were not trivial to interpret but, in the end, genuine Martian organics were identified. Interestingly, the organic matter was found in rocks formed about 3.5 billion years ago when Mars was drying out, although water persisted in Gale crater for thousands to millions of years more. The new finding shows that organic molecules located near the surface resist solar radiation. There is no compelling evidence for a biological origin of these molecules, but it persists as a legitimate working hypothesis. In case this hypothesis is true, one cannot tell if life had existed sometime in the past or if it is still there, somewhere.

A second study,12 published in the same month and in the same journal as the study on Gale crater organic matter, showed that methane, the simplest organic molecule (CH₄), in the Martian atmosphere has large seasonal variations, which can only be accounted for if small localized sources of methane are released from the Martian surface or subsurface reservoirs. In other words, there must be a “methane cycle.” Cycles of organic matter are amenable to life. Methane can be created by geological interaction between rocks, water, and heat, or it could be a product of microbes (or protomicrobes) that release waste methane. Again, there is no compelling evidence for life on Mars, past or present, but the opposite cannot be granted either, so more definite conclusions demand additional information from missions to Mars. At present, two new rovers are planned to be launched, one from NASA and one from the European Space Laboratory. The European will drill much deeper than Curiosity did. The next NASA rover will collect rocks that will be brought back to Earth on a later mission, so a more detailed analytic study will be possible.

Questions

1. Why is so much importance given to finding water?
2. Since there is methane, why couldn’t methane replace water in originating life?

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9 McKay DS et al (1996) Search for past life on Mars: possible relic biogenic activity in Martian meteorite ALH84001. Science 273:924–930.
10 Treiman A. Traces of ancient Martian life in meteorite ALH84001: An outline of states in late 2003. https://planetaryprotection.nasa.gov/summary/alh84001.
11 Anders E et al (1996) Evaluating the evidence of part life on Mars. Science 274:2119–2125.
12 Eigenbrode JL et al (2018) Organic matter preserved in 3-billion-year-old mudstones at Gale crater, Mars. Science 360:1096–1101.
3. Silicon is abundant on Mars. Why is it not reasonable to assume it may replace carbon in the process of originating life?

**Biochemical Insight**

1. The origin of life on Earth is unconceivable without liquid water. The elemental composition of Mars is not radically different from that of Earth. Silicon, oxygen, iron, calcium, and potassium are abundant in the crust. The red color of the planet is due to iron oxides. Hydrogen is present as water ice, whereas carbon occurs mainly as carbon dioxide in the atmosphere and, depending on climate seasons, as dry ice (solid carbon dioxide) at the poles. The atmosphere also has molecular nitrogen. The chemistry of these elements demands a medium (solvent) that enables and facilitates reactions; otherwise, chemical evolution cannot take place. The present climate of Mars is too cold for liquid water, but the occurrence of liquid water in the past is a hypothesis not yet excluded.

2. At Earth’s atmospheric pressure, methane is liquid at temperatures below −162 °C, which is too extreme for the origin of life as chemical reactions are slowed down at low temperatures. Moreover, methane is not polar and cannot participate in H-bonding sharing. See Chap. 2 for more details on this subject.

3. Silicon has an atomic number of 14 and has chemical properties similar to carbon. Silicon however has high affinity for oxygen. C–O bond energy is −360 kJ/mol, while Si–O is 452 kJ/mol. Also, Si–Si bond energy is 226 kJ/mol, in contrast with 356 kJ/mol for the C–C bond. Overall, these figures show that compounds having Si–Si multiple bonds are unstable compared to compounds based on C–C scaffolds, and silicates (Si–O rich compounds) are very stable, which is why Si is deposited in minerals in the crust of Earth and Mars and life based on C–C scaffolds cannot translate to Si–Si scaffolds.

**Final Discussion**

**Astrobiology**

Controversy and speculation based on misinterpretation or overinterpretation of data, such as occurred in the public debate of possible life signs in ALH84001, has led to the need to carry out robust, unbiased, interdisciplinary work to achieve evidence-based irrefutable conclusions in the field of the potential for life to exist beyond the Earth. Astrobiology emerged and consolidated in this context, gathering contributions from astronomers, physicists, chemists, biochemists, geologists, climatologists, etc. On Mars, astrobiological activities have evolved from “follow the water” approach to a “seek the signs of life” strategy, in which evidence of cells preserved in rocks is researched for. However, in hundreds of millions of years, these eventual
fossils may have been destroyed. For this reason, astrobiology is now focused in evidence that may come from shielded regions well beneath the surface of the Red Planet.

**Extraterrestrial Exporting of Life**

Microbe resistance to extreme environments is sometimes amazing. Even in extreme conditions of pH, temperature, and pressure, some species of microbes thrive and develop. Surprisingly, some forms of life have been found in the stratosphere, in spite of being a dry, cold, and low pressure (hypobaric) environment severely irradiated with UV, such as is on ... Mars. So, it is reasonable to assume Terrestrial microbial life may persist on Mars, leading to colonization in the event cells that are transported by human devices to Mars. It is not likely that microbes on the surface of spacecrafts can survive to exposure to cosmic rays, but the competence of some microbes to repair radiation-caused damages is surprising (see Box 2.2), and the existence of pockets of microbes shielded in niche locations during traveling cannot be excluded. Naturally, survival during the trip does not guarantee conditions to grow and multiply in other planets, but the risk of colonization does exist to some extent. Above all, this issue raises the need for awareness of the biochemistry of extremophiles on Earth and the need to account for co-colonization of other planets with Earth microbes in case of human presence, which is the reverse of the novel by H.G. Wells, *The War of the Worlds* (see Challenging Case 1.1): Reality is not about microbes protecting the Earth, but about microbes being potential biological assault weapons on other planets.

Close to our planet, the “pristine environment” of the moon may have already been broken with a multicellular complex form of life: an Israeli spacecraft that crash-landed on the moon in April 2019 was carrying Tardigrades—often called water bears or moss piglets—which are creatures under a millimeter long that can survive being heated to 150 °C, frozen at very low temperatures, or being dehydrated for long periods. When dried out, they retract their heads and their eight legs, and shed almost all of the water in their body, which makes their metabolism slow tremendously. If reintroduced to water decades later, they are able to reanimate. The survival of Tardigrades is questionable, and the biological impact of the crash of the spacecraft, if any, is yet to be seen but, as BBC reporters wrote in good humor: “And alternatively, there is definitely some great source material for a sci-fi/horror movie. Attack of the Moss Piglets from the Moon? We’d watch it.”

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