Chapter 1
Introduction: Life Is Made of Molecules!

Studying molecules is the key to understanding 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 sufficient 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" involving 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 and 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 is 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 elucidated 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,

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DOI 10.1007/978-1-4939-3058-6_1

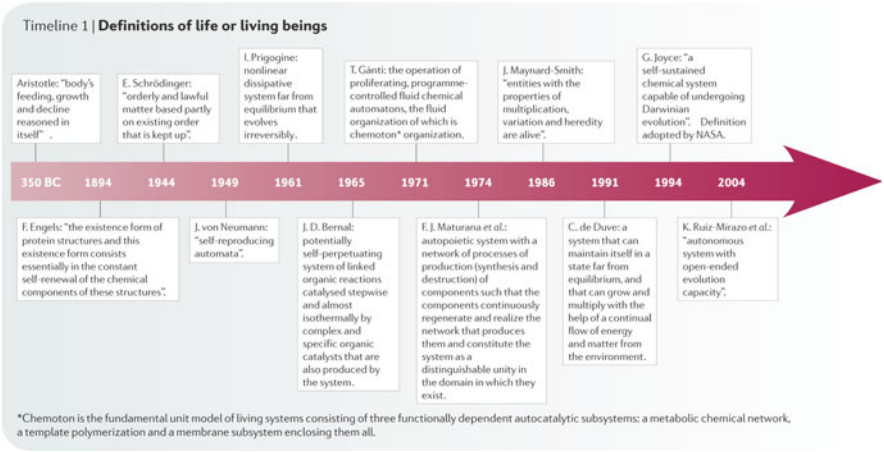
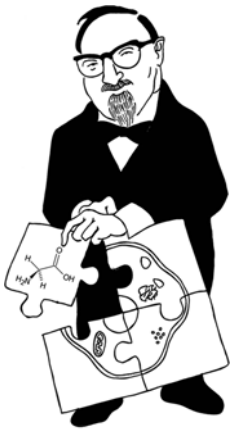


Fig. 1.1 Timeline for the definitions of life or living beings. Figure reproduced with permission from Moreva & López-Garcia, Nat. Rev. Microbiol. 7:306–311, 2009

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 compartmentalization. The replicator polymer is essential to transmit the molecular information inherited from the previous generation, and a membrane 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 details and chronological order of events that resulted in cells as we know today is far from being established.



Aleksander Oparin (1894 - 1980)

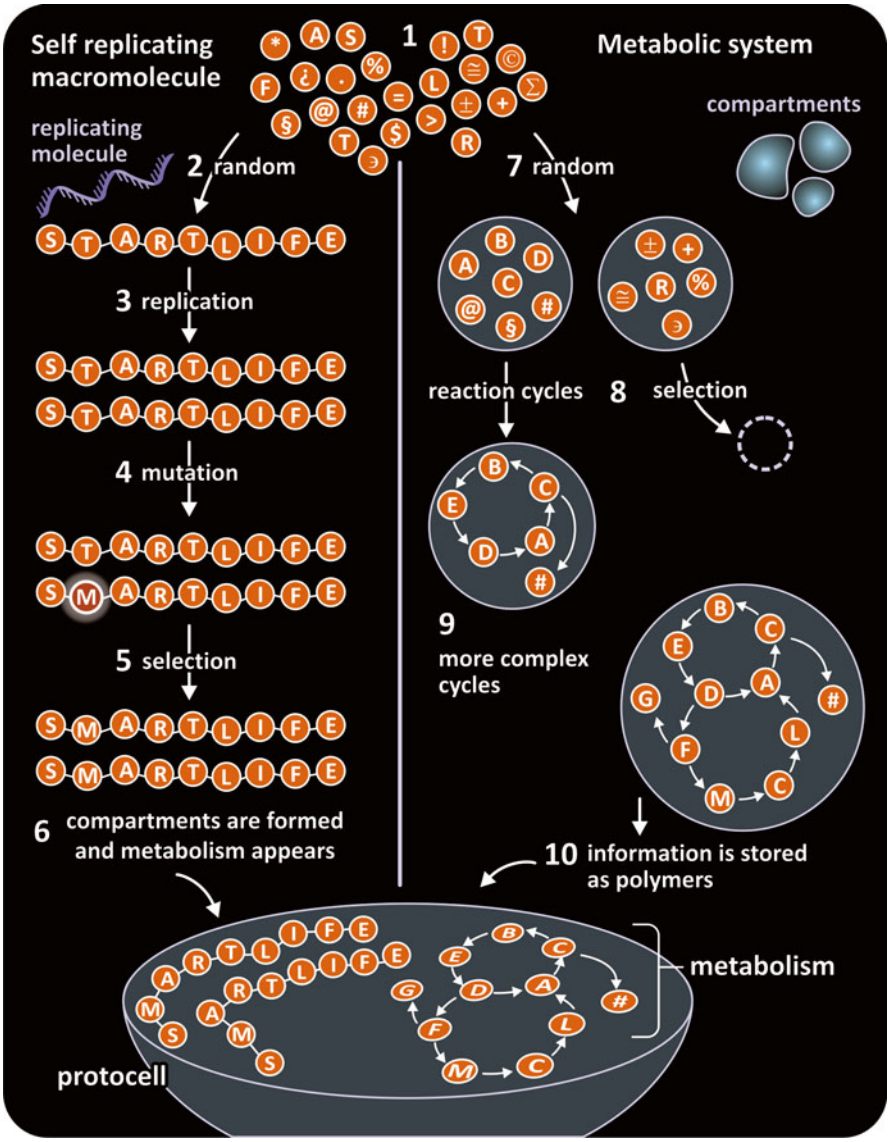


Fig. 1.2 Schematic representation of the replicator and metabolism hypotheses to describe the origin of life. Both models are molecular in nature and agree on the critical roles of a replicator molecule and compartmentalization but differ on the sequence of events. Figure reproduced with permission from Saphiro, Investigacion y Ciencia 371, 2007

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. It 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 or dengue virus, have RNA genomes). Concomitantly, RNA conformation dynamics enables catalytic activity, a perfect combination for the original replicator. The introduction of mutations and other errors in replication, in addition to other mechanisms, 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 cause 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 in not dependent on a starting event that is nearly impossible to succeed. The key process was the confinement of small molecules that reacted among them. 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.

Nowadays, virtually all cell membranes are formed non-exclusively but mostly by lipids. Modern 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? Orevices in the outer layers of rocks are a possibility. Phospholipids or other surfactant molecules may have started as coatings that, due to their intrinsic dynamics and capability to expand into a film and seal, may have evolved into membranes. Lipids and other surfactants have the ability to form three-dimensional structures other than lamellae that may have contributed to confine chemical systems (Fig. 1.3).

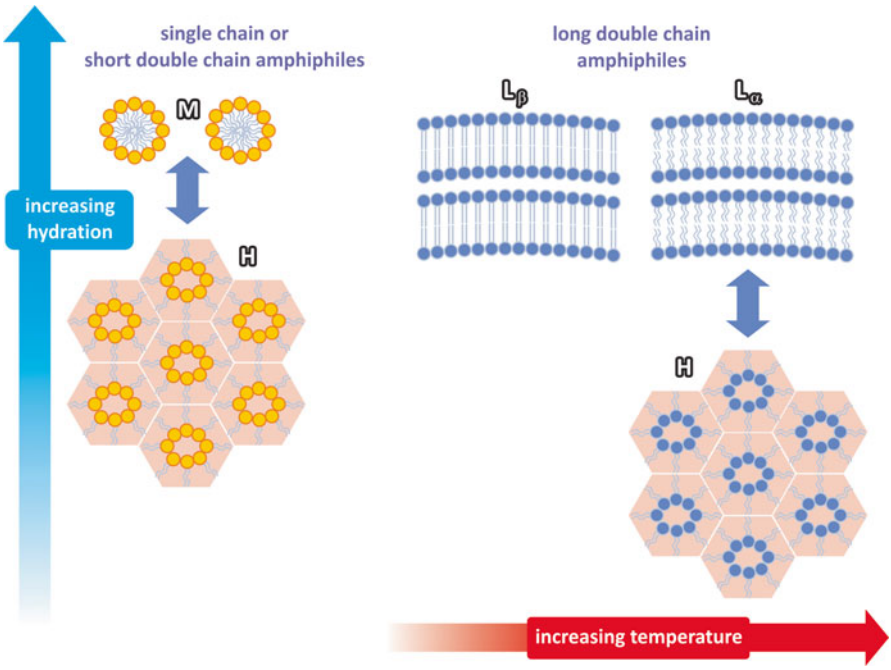


Fig. 1.3 The structure of lipid assemblies depends mainly on the degree of hydration and the molecular structure of lipids. Lipids may organize in different ways: rigid bilayers (L_β), fluid bilayers (L_α), micelles (M), or hexagonal (H) phases

Metabolism evolved towards self-regulation creating homeostasis, a situation whereby a balance exists. Small to moderate perturbations of such balance 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).

Nowadays, even the simplest cells, mycoplasma bacteria, for example, 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 "metabolic maps" showing the main metabolic sequences in living beings can be drawn (Fig. 1.5). It is amazing that these complex series of reactions operate and do not conflict with each other. In reality, not all reactions depicted in (Fig. 1.5) occur in the same species and the ones that do occur in the same species may not be present in all cells. In case they co-exist in the same cell, they may not occur in the

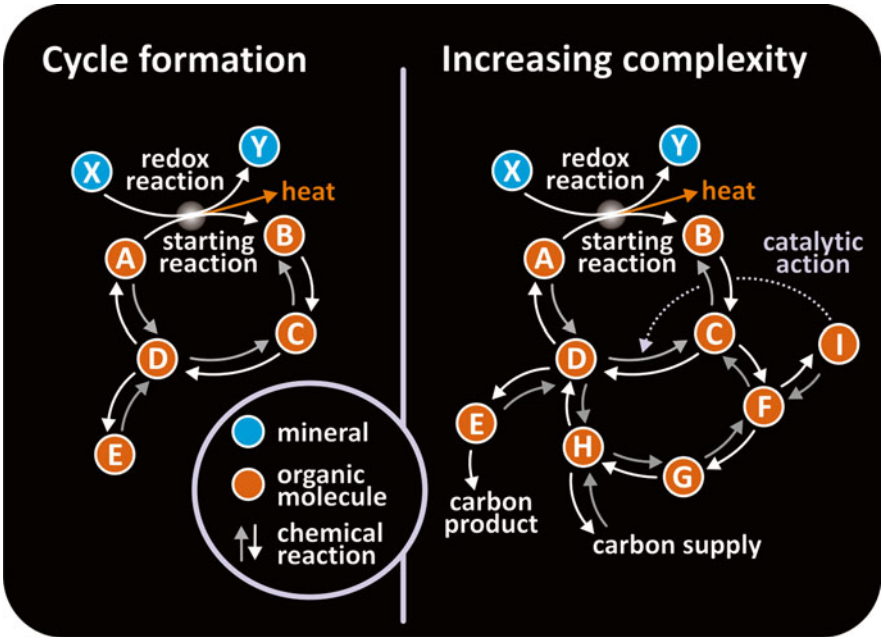


Fig. 1.4 The evolution of networks of chemical reactions. Simple cycles of reactions (*left*) may have evolved in complexity (*right*). The interference of certain metabolites on the course of reactions possibly resulted in self-regulated metabolisms. Figure reproduced with permission from Saphiro, Investigacion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

same cell compartment and in case they do, they may not be working at the same time. "Complex" does not mean "confusing."

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 puzzle be explained? Essentially, 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.

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

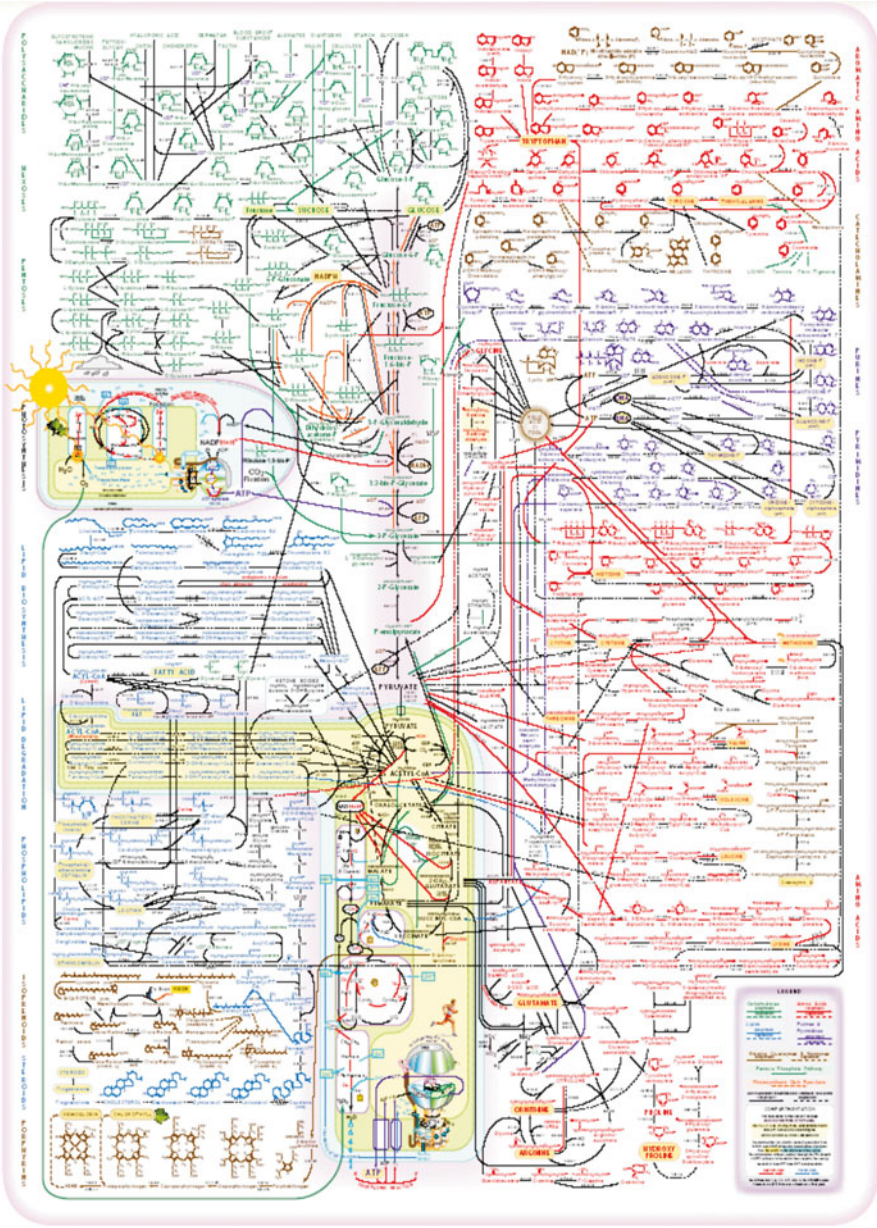


Fig. 1.5 A metabolic map showing a hypothetical cell, where the whole metabolism would gather many different sectorial metabolisms: amino acids, phospholipids, steroids, lipids, saccharides, etc. In reality, not all cells perform all sectorial metabolisms; those that occur in a certain cell may not occur in the same organelle and those that occur in the same organelle may not occur at the same time. The metabolism as a whole is usually so complex that in practice one tends to refer to "metabolisms" referring to 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. Figure reproduced with permission of IUBMB, International Union of Biochemistry and Molecular Biology

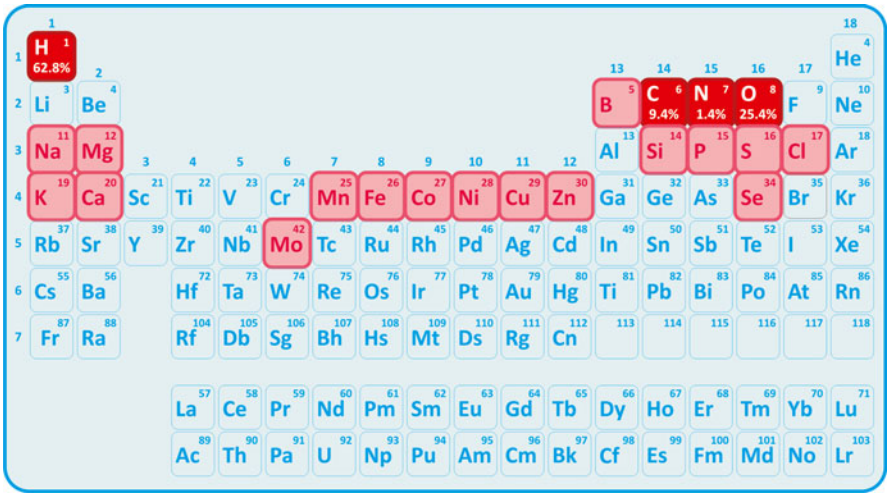


Fig. 1.6 Periodic chart of the elements, stressing the abundance of some in living beings (highlighted *red*). It should be noticed that very few elements are needed to "build" almost the totality of cells, and some elements are only present in trace amounts (highlighted *pink*). Yet, the elements that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

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 boundary between living and non-living, 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, it is 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 as 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 their own machinery of molecular synthesis.

Virus–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 or 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, the influenza A virus being the major cause of seasonal flu). Influenza A virus is an enveloped virus, whose genome consists of eight single-stranded RNAs that encode

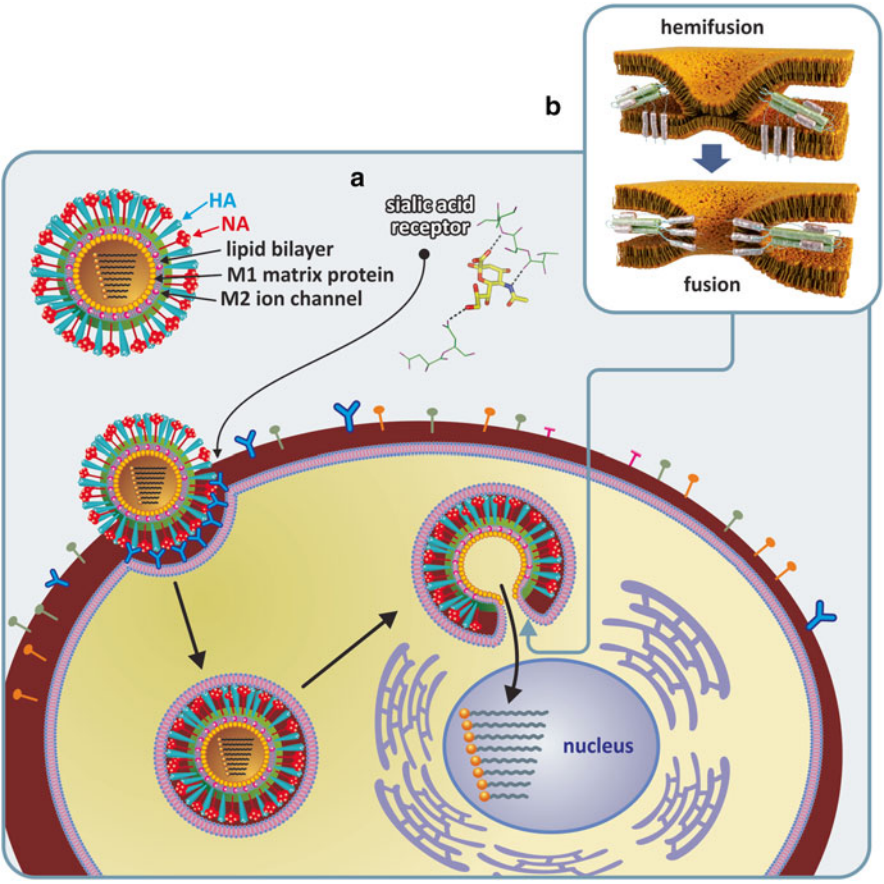


Fig. 1.7 Influenza virus entering a cell having sialic acid-containing receptor on its surface. The orientation, chemical nature, and distance of the binding amino acids of hemagglutinin A (HA) are such that sialic acid is able to engage in hydrogen bonds and other attractive forces. Panel (**a**) shows a zooming of the part of HA protein backbone contacting the sialic acid (protein carbon backbone in *green*; sialic acid carbon backbone in *yellow*). Upon acidification of endocytic vesicles inside the cell, HA undergoes conformational changes (not shown) that bring viral and cellular membranes in contact (Panel (**b**)) leading to the collapse of the membranes (named fusion) from which the viral content is released inside the cell

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 cell surface, which are known as 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 atoms 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, to the endocytic vesicle membrane. Entropy balance then serves as a driving force (see Sect. 3.1) that promotes the fusion peptide 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 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 strains infect the same cell, the RNA of both may coexist in the nucleus. Scrambling of RNA originates new virus having random mixtures of the genetic material of both strains. The combined viruses may not be functional but occasionally a new strain of increased efficacy 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 hundreds of millions (!) of people across Europe and the USA (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.

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,

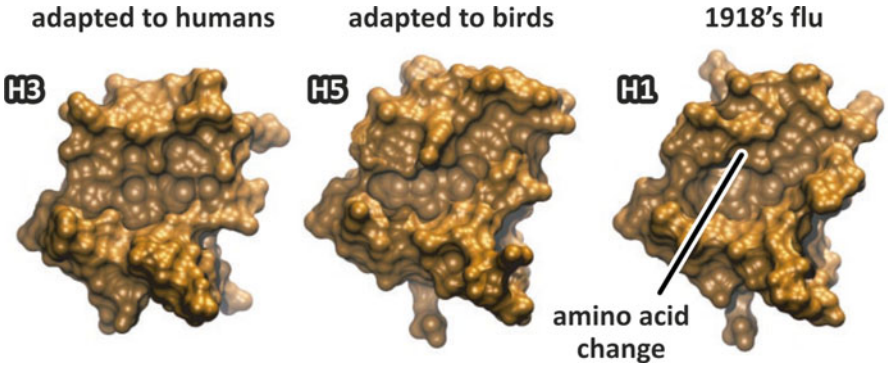


Fig. 1.8 Hemagglutinin 3 is adapted to human cells; Hemagglutinin 5 is adapted to birds. In 1918, a mutation in a single amino acid in the binding site of bird hemagglutinin made it able to bind human cells having sialic acid on its surface. This caused a tragic pandemic of flu among humans—the "Spanish flu" or "1918 flu." Figure reproduced with permission from Stevens et al., Science 303:1866–1870, 2004

Box 1.1: "Spanish Flu," Terrible and Almost Forgotten

Between April 1918 and February 1919, the world suffered the most severe pandemics 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 to 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 to 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 pandemics, 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 just 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 to pneumonia, which was a consequence of opportunistic infection of the lungs by bacteria. The histological properties of the lungs were transformed, and there was accumulation of fluids that literally suffocated the victims, just as if they were drowning.

(continued)

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 are still a challenge. The quest for the sequence of amino acid residues 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 the Hultin 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 suffered 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 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.

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Box 1.1: (continued)

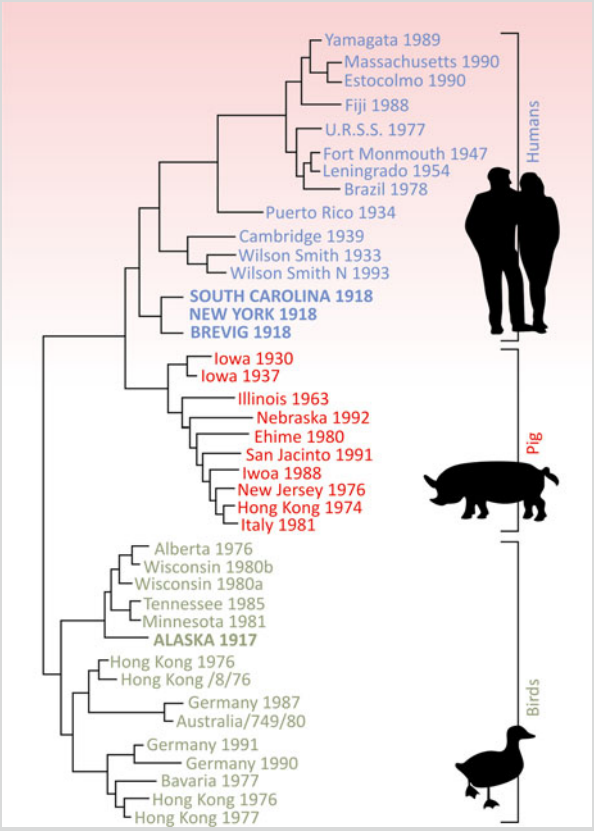

Figure reproduced with permission from Taubenberger et al., Temas Investigacion y Ciencia 48, 2007

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 same target or in any other molecule of the body. The uniqueness of the binding site is directed 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 Walls 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 basis of these principles (Fig. 1.9). In addition to the efficacy in binding to its target, drug

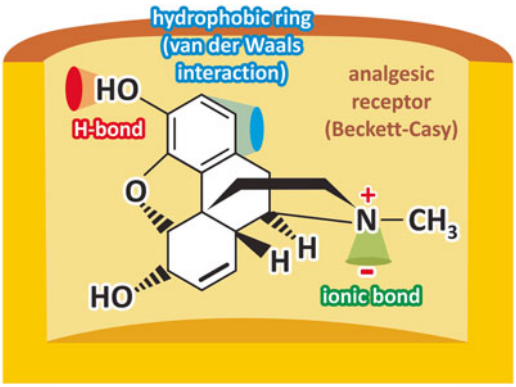

Fig. 1.9 Beckett–Casy hypothesis for the binding of an analgesic molecule (such as morphine, which is illustrated) to an opioid receptor. While the exact structure of the receptor is not known, the key interaction/attraction forces were identified: electrostatic attraction, H-bonds, and van der Waals 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

molecules cannot be exceedingly toxic or have significant other undesired effects, which are directly related to its reactivity and selectivity.

The same reasoning applies to complex therapeutic molecules such as antibodies. Let us now take one of the antibodies that target the protein gp120 on the surface of the human immunodeficiency virus (HIV) (Fig. 1.10). This protein is responsible for the binding to the receptors and co-receptors of the T lymphocytes, this being the first step of infection in acquired immune deficiency syndrome (AIDS). 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 targets, but the overall number of bonds (hydrogen bonding, electrostatic interactions, van der Waals interactions, etc.) involved may be higher, resulting in extreme selectivity and very strong binding forces.

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

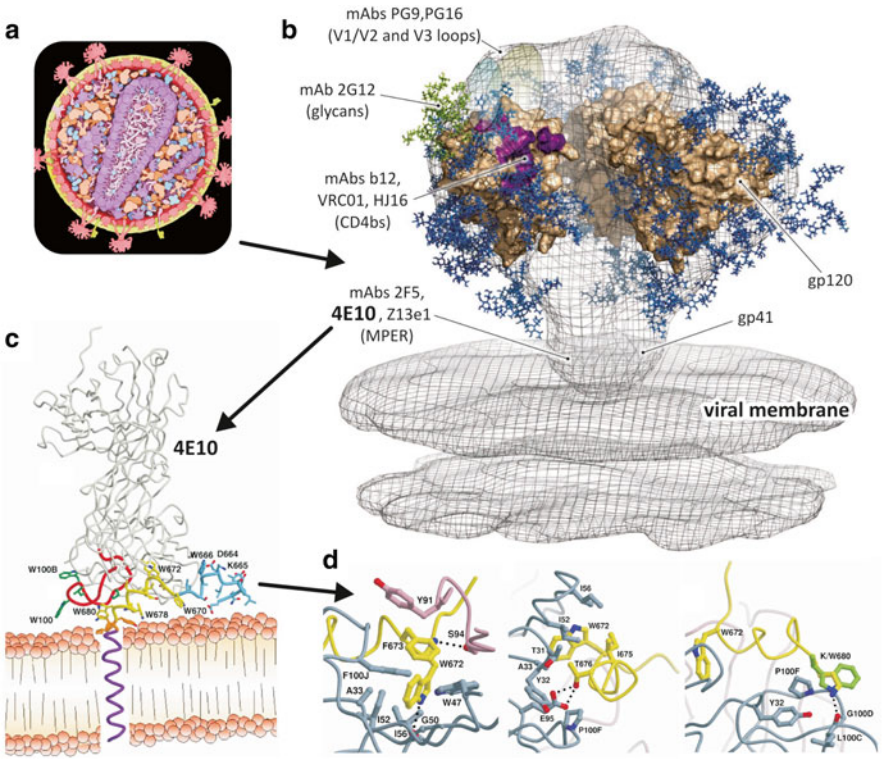

Fig. 1.10 (**a**) Illustration showing HIV particle, highlighting its capsid (*magenta*) surrounding viral nucleic acid and the envelope proteins (*pink*). Besides the capsid, the virion is loaded with several other proteins with different functions in the replication cycle (reproduced with permission from Goodsell, The Machinery of Life, 2009). (**b**) Broadly neutralizing monoclonal antibodies target specific motifs (epitopes) at the surface of the envelope proteins, gp120 and gp41. The model depicted was generated gathering scientific data from different sources. The contour of the envelope proteins and viral membrane is shown in *gray*; what is known from the structure of gp120 is shown in *colors*. The glycosidic (saccharidic) part of the protein is shown in *green* and *blue*. MPER stands for Membrane Proximal External Region and refers to the proteic part of gp41 nearest to the viral membrane (reproduced with permission from Burton & Weiss, Science 239:770–772, 2010). (**c**, **d**) Some antibodies, such as 4E10, target this region of the protein. At the contact points between the amino acid residues of antibody and gp41, attractive forces such as ionic, H-bonds, and van der Waals interactions contribute to a strong binding. The chemical nature and the spatial arrangement of the amino acids confer selectivity to antibody–epitope interaction. Figure reproduced with permission of Elsevier from Cardoso et al., Immunity, 22:163–173, 2005

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 five).

The preclinical development in the first step is the development stage and the last step before clinical trials. Preclinical studies consist in as many experiments in vivo

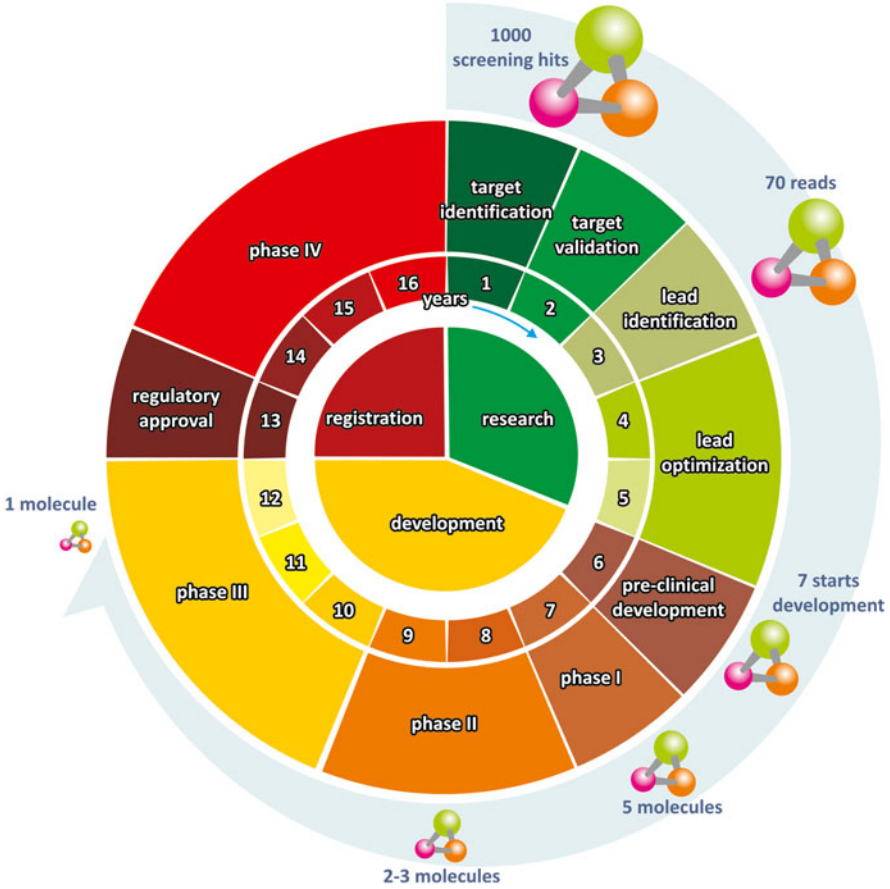

Fig. 1.11 The drug discovery and development process, generally termed "pipeline" in pharmaceutical industries. There are three main stages, research, development, and registration (*center*), distributed over several years (numerical timeline). Each stage is divided in subphases. During research phase, relevant targets for selected pathologies are identified and molecules to interfere with those targets are selected ("leads"). Research stage takes typically around 5 years. The development phase 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, there is evaluation of results; safety and/or efficacy issues may prevent further tests. Typically, for each nearly 1000 molecules starting the process, only one ends the last phase successfully. This ratio, named "attrition rate," is incredibly low. 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)

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 administrating 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 Phase I clinical trials, it is safety that is tested, using conservative doses of the compounds under study. Drug 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 in up to several hundred individuals for several weeks or 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 conclusions is, by itself, a complex discipline.

Phase III clinical trials are a replica of Phase II, 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.

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

(continued)

Box 1.2 (continued)
discovered a healing fluid that could "cure" many ailments. Mesmer became highly sought after in Paris, where he would routinely "mesmerize" his followers—one of whom was Marie Antoinette. The King asked a commission of the French Academy of Sciences to look into the claims. (The members included the chemist Antoine Lavoisier, and Joseph Guillotin—who invented the device that would eventually separate the King's head from his body.) The commission replicated some of Mesmer's sessions and, in one case, asked a young boy to hug magnetized trees that were presumed to contain the healing powers invoked by Mesmer. He did as directed and responded as expected: he shook, convulsed, and swooned. The trees, though, were not magnetic, and Mesmer was denounced as a fraud. Placebos and lies were intertwined in the public mind.

It was another 150 years before scientists began to focus on the role that emotions can play in healing. During the World War II, Lieutenant Colonel Henry Beecher met with more than 200 soldiers, gravely wounded but still coherent enough to talk; he asked each man if he wanted morphine. Seventy-five percent declined. Beecher was astounded. He knew from his experience before the war that civilians with similar injuries would have begged for morphine, and he had seen healthy soldiers complain loudly about the pain associated with minor inconveniences, like receiving vaccinations. He concluded that the difference had to do with expectations; a soldier who survived a terrible attack often had a positive outlook simply because he was still alive. Beecher made a simple but powerful observation: our expectations can have a profound impact on how we heal.

There is also a "nocebo effect." Expecting a placebo to do harm or cause pain makes people sicker, not better. When subjects in one notable study were told that headaches are a side effect of lumbar puncture, the number of headaches they reported after the study was finished increased sharply.

For years, researchers could do little but guess at the complex biology of the placebo response. A meaningful picture began to emerge only in the 1970s, with the discovery of endorphins, endogenous analgesics produced in the brain.

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 is 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 to 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 vitro tests, from which only 70 are selected as leads, which are then optimized to form 7 drug candidates that enter clinical trials. Out of these 7, only 2–3 reach Phase III clinical trials and only 1 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 (number 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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