"George Church is one of the most brilliant scientists in the world."

—STEVEN PINKER

REGENESIS

How Synthetic Biology Will Reinvent Nature and Ourselves

GEORGE CHURCH AND ED REGIS

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PROLOGUE

FROM BIOPLASTICS TO *H. SAPIENS* 2.0

In December 2009, patrons of the John F. Kennedy Center for the Performing Arts in Washington, DC, experienced a mild jolt of biological future shock when their pre-performance and intermission drinks—their beers, wines, and sodas—were served to them in a new type of clear plastic cup. The cups looked exactly like any other transparent plastic cup produced from petrochemicals, except for a single telling difference: each one bore the legend, "Plastic made 100% from plants."

Plants?

Indeed. The plastic, known as Mirel, was the product of a joint venture between Metabolix, a Cambridge, Massachusetts, bioengineering firm, and Archer Daniels Midland, the giant food processing company that had recently constructed a bioplastics production plant in Clinton, Iowa. The plant had been designed to churn out Mirel at the rate of 110 million pounds per year.

Chemically, Mirel was a substance known as polyhydroxybutyrate (PHB), which was normally made from the hydrocarbons found in petroleum. But starting in the early 1990s, Oliver Peoples, a molecular biologist who was a cofounder of Metabolix, began looking for ways to produce polymers like PHB by fermentation, by the action of genetically altered microbes on a feedstock mixture.

After seventeen years of research and experimentation (and having been laughed out the doors of several chemical companies), Peoples had developed an industrial strain of a proprietary microbe that turned corn sugar into the PHB plastic polymer. In its broadest outlines, the process was not all that different from brewing beer, which was also accomplished by fermentation: microorganisms (yeast cells) acted on malt and hops to produce ethanol. In the case of Mirel, the microbial fermentation system consisted of a large vat that combined the engineered microbes with corn sugar and other biochemical herbs and spices. The microbes metabolized the corn sugar and turned it into bioplastic, which was then separated from the organisms and formed into pellets of Mirel. Ethanol was a chemical, and so was PHB, but in both cases microbes effected the transformation of organic raw material into a wholly different kind of finished product.

The microbial-based PHB had some key environmental advantages over the petrochemical-derived version. For one thing, since it wasn't made from petroleum, it lessened our dependence on fossil fuels. For another, its chief feedstock material, corn, was an agriculturally renewable and sustainable resource, not something we were going to run out of any time soon. For a third, Mirel bioplastic resins were the only nonstarch bioplastics certified by Vinçotte, an independent inspection and certification organization, for biodegradability in natural soil and water environments, such as seawater. If any of the plastic cups used at the Kennedy Center ended up in the Potomac River, they would break down and be gone forever in a matter of months. (Biodegradation is not necessarily the panacea it was once thought to be, since it releases greenhouse gases, while non-degradation, ironically, sequesters carbon.)

Constructing a microbe that would convert corn into plastic, in a process akin to beer

brewing, was just one example of the transformations made possible by the emerging discipline of synthetic biology—the science of selectively altering the genes of organisms to make them do things that they wouldn't do in their original, natural, untouched state.

But the feat of turning corn into plastic was merely the tip of the synthetic biology iceberg. By the first decade of the twenty-first century microbe-made commodities were yielding up products that nobody would have guessed were manufactured by bacteria in three-story-high industrial vats. Carpet fibers, for example.

In 2005 Mohawk Industries introduced its new SmartStrand carpet line. It was based on the DuPont fiber Sorona, which was made out of "naturally occurring sugars from readily available and renewable crops." The Sorona fiber had a unique, semicrystalline molecular structure that made it especially suitable for clothing, automobile upholstery, and carpets. The fiber had a pronounced kink in the middle, and the shape acted as a molecular spring, allowing the strands to stretch or deform and then automatically snap back into their original shape. That attribute was perfect for preventing baggy knees or elbows, or for making carpets that were highly resilient, comfortable, and supportive.

Sorona's main ingredient was a chemical known as 1,3-propanediol (PDO), which was classically derived from petrochemicals and other ingredients that included ether, rhodium, cobalt, and nickel. In 1995 DuPont had teamed up with Genencor International, a genetic engineering firm with principal offices in Palo Alto, to research the possibility of producing PDO biologically. Scientists from the two companies took DNA from three different microorganisms and stitched them together in a way that resulted in a new industrial strain of the bacterium *Escherichia coli*. Specifically, they programmed twenty-six genetic changes into the microbe enabling it to convert glucose from corn directly into PDO in a fermenter vat, like beer and Mirel.

In 2003 DuPont trademarked the name Bio-PDO and started producing the substance in quantity. The company claimed that this was the first time a genetically engineered organism had been utilized to transform a naturally occurring renewable resource into an industrial chemical at high volumes. The US Environmental Protection Agency, which regarded Bio-PDO as a triumph of green chemistry, gave DuPont the 2003 Greener Reaction Conditions Award (a part of the Presidential Green Chemistry Challenge). And why not? The biofiber used greener feedstocks and reagents, and its synthesis required fewer and less expensive process steps than were involved in manufacturing other fibers. The production of Sorona consumed 30 percent less energy than was used to produce an equal amount of nylon, for example, and reduced greenhouse gas emissions by 63 percent. For its part, Mohawk touted its Sorona carpeting as environmentally friendly: "Every seven yards of SmartStrand with DuPont Sorona saves enough energy and resources to equal one gallon of gasoline—that's 10 million gallons of gasoline a year!" Here it was, finally: the politically correct carpet.

What these examples hinted at, however, was something far more important than mere political correctness, namely, that biological organisms could be viewed as a kind of high technology, as nature's own versatile engines of creation. Just as computers were universal machines in the sense that given the appropriate programming they could simulate the activities of any other machine, so biological organisms approached the condition of being universal constructors in the sense that with appropriate changes to their genetic programming, they could be made to produce practically any imaginable artifact. A living organism, after all, was a ready-made, prefabricated production system that, like a computer, was governed by a program, its genome. Synthetic biology and synthetic genomics, the large-scale remaking of a genome, were attempts to capitalize on the facts that biological organisms are programmable manufacturing systems, and that by making small changes in their genetic software a bioengineer can effect big changes in their output. Of course, organisms cannot manufacture just anything, for like all material objects and processes they are limited and circumscribed by the laws of nature. Microbes cannot convert lead into gold, for example. But they can convert sewage into electricity.

This astonishing capacity was first demonstrated in 2003 by a Penn State team headed by researcher Bruce Logan. He knew that in the United States alone, more than 126 billion liters of wastewater was treated every day at an annual cost of $25 billion, much of

it spent on energy. Such costs, he thought, “cannot be borne by a global population of six billion people, particularly in developing countries.” It was widely known that bacteria could treat wastewater. Separately, microbiologists had known for years that bacteria could also generate electricity. So far, nobody had put those two talents together. But what if microbes could be made to do both things simultaneously, treating wastewater while producing electrical energy?

Key to the enterprise would be the microbial fuel cell—a sort of biological battery. In ordinary metabolism, bacteria produce free electrons. A microbial fuel cell (MFC) consists of two electrodes—an anode and a cathode. A current is set up between them by the release of electrons from bacteria in a liquid medium. Electrons pass from the bacteria to the anode, which is connected to the cathode by a wire.

Logan and his colleagues constructed a cylindrical microbial fuel cell, filled it with wastewater from the Penn State water treatment plant, and then inoculated it with a pure culture of the bacterium *Geobacter metallireducens*. Lo and behold, in a matter of hours the microbe had begun purifying the sewage while at the same time producing measurable amounts of electricity. These results “demonstrate for the first time electricity generation accompanied by wastewater treatment,” Logan said. “If power generation in these systems can be increased, MFC technology may provide a new method to offset wastewater treatment operating costs, making advanced wastewater treatment more affordable for both developing and industrialized nations.”

The general setup wasn’t difficult to replicate and within a few years a sophomore at Stuyvesant High School in New York City, Timothy Z. Chang, was designing, building, and operating microbial fuel cells at home and in his high school lab. He had experimented with some forty different strains of bacteria to discover which was best suited to maximum electricity production. “It may be possible to achieve even higher power yields through active manipulation of the microbial population,” he wrote in a formal report on the project.

By 2010 several teams of researchers were working on scaling up bacterial electricity production from sewage to make it into a practical, working, real-world option. By this time, synthetic biologists had gotten microbes to perform so many different feats of creation that it was clear that many of nature’s basic units of life—microbes—were undergoing an extreme DNA makeover, a major course of redesign from the ground up. Engineered microbes produced diesel oil, gasoline, and jet fuel. Microbes were made to detect arsenic in drinking water at extremely low concentrations (as low as 5 parts per billion) and report the fact by changing color. There were microbes that could be spread out into a biofilm. By producing a black pigment in response to selective illumination, they could copy superimposed patterns and projected images—in effect, microbial Xerox machines.

A student project reprogrammed *E. coli* bacteria to produce hemoglobin (“bactoblood”), which could be freeze-dried and then reconstituted in the field and used for emergency blood transfusions. In 2006, just for fun, five MIT undergrads successfully reprogrammed *E. coli* (which as a resident of the intestinal tract smelled like human waste) to smell like either bananas or wintergreen.

E. coli was so supple, pliable, and yielding that it seemed to be the perfect biological platform for countless bioengineering applications. One of its greatest virtues was that the *E. coli* bacterium (and cousins, the Vibrio) are the world’s fastest machines at doubling, small or large.* It reproduced itself every twenty minutes, so that theoretically, given enough simple food and stirring, a single particle of *E. coli* could multiply itself exponentially into a mass greater than the earth in less than two days.

Still, as malleable as it was, University of Wisconsin geneticist Fred Blattner decided he could materially improve the workhouse K-12 strain of the microbe to make it an even better chassis for synthetic biology engineering projects. The microbe had some 4,000 genes; many had no known function, while others were nonessential, redundant, or toxic. So Blattner stripped 15 percent of its natural genes from the K-12 genome, making it a sort of reduced instruction set organism, a streamlined, purer version of the microbe. Blattner described it as “rationally designed” and said that his genetic reduction

"optimizes the *E. coli* strain as a biological factory, providing enhanced genetic stability and improved metabolic efficiency." With forty genome changes, he had pre-engineered the microbe in order to make it easier to engineer.

In 2002 Blattner founded Scarab Genomics to sell his new and improved organism, now billing it as "Clean Genome *E. coli*" and marketing it under the slogan "Less is better and safer!" Researchers can buy quantities of the microbe, online or by fax, for as little as $89 a shot (plus a $50 shipping fee).

The upshot of all this is that, at least at the microbial level, nature has been redesigned and recoded in significant ways. Genomic engineering will become more common, less expensive, and more ambitious and radical in the future as we become more adept at reprogramming living organisms, as the cost of the lab machinery drops while its efficiency rises, and as we are motivated to maximize the use of green technologies.

Given the profusion and variety of biological organisms, plus the ability to reengineer them for a multiplicity of purposes, the question was not so much what they can be made to do but what they can't be made to do, in principle. After all, tiny life forms, driven solely by their own natural DNA, have, just by themselves, produced large, complex objects: elephants, whales, dinosaurs. A minuscule fertilized whale egg produces an object as big as a house. So maybe one day we can program an organism, or a batch of them, to produce not the whale but the actual house. We already have bioplastics that can be made into PVC plumbing pipes; biofibers for carpeting; lumber, nature's own building material; microbe-made electricity to provide power and lighting; biodiesel to power the construction machinery. Why can't other microbes be made to produce whatever else we need?

In 2009 Sidney Perkowitz, a physicist at Emory University in Atlanta with a special interest in materials science, was asked to speculate about the future of building materials. "Think about the science-fictionish possibility of bioengineering plants to produce plastic exactly in a desired shape, from a drinking cup to a house," he said. "Current biotechnology is far short of this possibility, but science fiction has a way of pointing to the future. If bioplastics are the materials breakthrough of the 21st century, houses grown from seeds may be the breakthrough of the 22nd."

Similar proposals have been made by others, and they may be much closer than the twenty-second century; for example, using modified gourds and trees to grow a primitive, arboreal house (inhabitat.com/grow-your-own-treehouse). The technology of determining the shape and chemical properties of plants by making them sensitive to simple cues of light and scaffolding is improving rapidly.

This focus on microbes and plants—especially on the overworked *E. coli* bacterium—may give rise to the impression that synthetic biology and genomic engineering have little to offer the charismatic megafauna—the higher organisms such as people. Nothing could be further from the truth. In fact these technologies have the power to improve human and animal health, extend our life span, increase our intelligence, and enhance our memory, among other things.

The idea of improving the human species has always had an enormously bad press, stemming largely from the errors and excesses associated with the eugenics movements of the past. Historically, eugenics has covered everything from selective breeding for the purpose of upgrading the human gene pool to massive human rights violations against classes of people regarded as undesirable, degenerate, or unfit because of traits such as religion, sexual preference, handicap, and so on, culminating, in the extreme case, in the Nazi extermination program.

Some proposals for enhancing the human body have had a harebrained ring to them, as for example the idea of equipping people with gills so that they could live in the sea alongside sharks. Burdened with past evils and silliness, any new proposal for changing human beings through genomic engineering faces an uphill battle. But consider this

modest proposal: What if it were possible to make human beings immune to all viruses, known or unknown, natural or artificial? No more viral epidemics, influenza pandemics, or AIDS infections.

Viruses do their damage by entering the cells of the host organism and then using the cellular machinery to replicate themselves, often killing the host cells in the process. This leads to the release of new viruses that proceed to infect other cells, which in turn produce yet more virus particles, and so on. Viruses can take control of a cell's genetic machinery because both the virus and the cell share the same genetic code. However, changing the genetic code of the host cell, as well as that of the cellular machinery that reads and expresses the viral genome, could thwart the virus's ability to infect cells (see Chapter 5).

All this may sound wildly ambitious, but there is little doubt that the technology of genome engineering is in principle up to the task. An additional benefit of engineering a sweeping multivirus resistance into the body is that it would alleviate a common fear concerning synthetic biology—the accidental creation of an artificial supervirus to which humans would have no natural immunity.

Genomic technologies can actually allow us to raise the dead. Back in 1996, when the sheep Dolly was the first mammal cloned into existence, she was not cloned from the cells of a live animal. Instead, she was produced from the frozen udder cell of a six-year-old ewe that had died some three years prior to Dolly's birth. Dolly was a product of nuclear transfer cloning, a process in which a cell nucleus of the animal to be cloned is physically transferred into an egg cell whose nucleus had previously been removed. The new egg cell is then implanted into the uterus of an animal of the same species, where it gestates and develops into the fully formed, live clone.

Although Dolly's genetic parent had not been taken from the grave and magically resurrected, Dolly was nevertheless probably a nearly exact genetic duplicate of the deceased ewe from which she had been cloned, and so in that sense Dolly had indeed been "raised from the dead." (Dolly was certainly different in the details of how the genome played out develop-mentally [a.k.a. epigenetically] but not so different as to discourage subsequent success in a variety of agricultural and research species.)

But even better things were in the offing. A few years after Dolly, a group of Spanish and French scientists brought to life a member of an extinct animal species—the Pyrenean ibex, or bucardo, a subspecies of wild mountain goat whose few remaining members had been confined to a national park in northern Spain. The species had become extinct in January 2000, when the very last living member, a thirteen-year-old female named Celia, was crushed to death by a falling tree. Consequently the International Union for the Conservation of Nature (IUCN) formally changed the conservation status of the species from EW, which meant "extinct in the wild," to EX, which meant "extinct," period.

Extinction, supposedly, was forever.

But in the spring of 1999, Dr. Jose Folch, a biologist working for the Aragon regional government, had taken skin scrapings from Celia's ears and stored the tissue samples in liquid nitrogen in order to preserve the bucardo's genetic line. A few years later, in 2003, Folch and his group removed the nucleus from one of Celia's ear cells, transferred it into an egg cell of a domestic goat, and implanted it into a surrogate mother in a procedure called interspecies nuclear transfer cloning.

After a gestation period of five months, the surrogate mother gave birth to a live Pyrenean ibex. By any standard, this was an astonishing event. After being officially, literally, and totally extinct for more than two years, a new example of the vanished species was suddenly alive and breathing.

Not for long, however. The baby ibex lived for only a few minutes before dying of a lung condition. Still, those scant minutes of life were proof positive that an extinct species could be resurrected, not by magic or miracles but by science.

"Nuclear DNA confirmed that the clone was genetically identical to the bucardo's donor cells," the group wrote in its report on the project. "To our knowledge, this is the first animal born from an extinct subspecies."

Almost certainly, it will not be the last. The bucardo's birth involved a bit of genomic reprogramming because the egg cell that developed into the baby ibex had not been

fertilized by a sperm cell but rather by the nucleus of a somatic (body) cell. The nucleus and the egg cell had to be jump-started into becoming an embryo in a process known as electrofusion, which melds the two together.

A later technique under development in my Harvard lab will allow us to resurrect practically any extinct animal whose genome is known or can be reconstructed from fossil remains, up to and including the woolly mammoth, the passenger pigeon, and even Neanderthal man. One of the obstacles to resurrecting those and other long extinct species is that intact cell nuclei of these animals no longer exist, which means that there is no nucleus available for nuclear transfer cloning. Nevertheless, the genome sequences of both the wooly mammoth and Neanderthal man have been substantially reconstructed; the genetic information that defines those animals exists, is known, and is stored in computer databases. The problem is to convert that information—those abstract sequences of letters—into actual strings of nucleotides that constitute the genes and genomes of the animals in question.

This could be done by means of MAGE technology—multiplex automated genome engineering. MAGE is sort of a mass-scale, accelerated version of genetic engineering. Whereas genetic engineering works by making genetic changes manually on a few nucleotides at a time, MAGE introduces them on a wholesale basis in automated fashion. It would allow researchers to start with an intact genome of one animal and, by making the necessary changes, convert it into a functional genome of another animal entirely.

You could start, for example, with an elephant's genome and change it into a mammoth's. First you would break up the elephant genome into about 30,000 chunks, each about 100,000 DNA units in length. Then, by using the mammoth's reconstructed genome sequence as a template, you would selectively introduce the molecular changes necessary to make the elephant genome look like that of the mammoth. All of the revised chunks would then be reassembled to constitute a newly engineered mammoth genome, and the animal itself would then be cloned into existence by conventional interspecies nuclear transfer cloning (or perhaps by another method, the blastocyst injection of whole cells).

The same technique would work for the Neanderthal, except that you'd start with a stem cell genome from a human adult and gradually reverse-engineer it into the Neanderthal genome or a reasonably close equivalent. These stem cells can produce tissues and organs. If society becomes comfortable with cloning and sees value in true human diversity, then the whole Neanderthal creature itself could be cloned by a surrogate mother chimp—or by an extremely adventurous female human.

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Any technology that can accomplish such feats—taking us back into a primeval era when mammoths and Neanderthals roamed the earth—is one of unprecedented power. Genomic technologies will permit us to replay scenes from our evolutionary past and take evolution to places where it has never gone, and where it would probably never go if left to its own devices.

Today we are at the point in science and technology where we humans can reduplicate and then improve what nature has already accomplished. We too can turn the inorganic into the organic. We too can read and interpret genomes—as well as modify them. And we too can create genetic diversity, adding to the considerable sum of it that nature has already produced.

In 1903 German naturalist Ernst Haeckel stated the pithy dictum "Ontogeny recapitulates phylogeny." By this he meant that the development of an individual organism (ontogeny) goes through the major evolutionary stages of its ancestors (phylogeny). He based this aphorism on observations that the early embryos of different animals resembled each other and that, as they grew, each one seemed to pass through, or recapitulate, the evolutionary history of its species. (For example, the human embryo at one point has gill slits, thus replicating an evolutionary stage of our piscine past.)

While it is clear that embryos develop primitive characteristics that are subsequently lost in adults, Haeckel's so-called biogenetic law is an over-statement and was not universally true when first proposed or today. However, I hereby propose a biogenetic law of my own, one that describes the current situation in molecular engineering and biotechnology: "Engineering recapitulates evolution." Through human ingenuity, and by using the knowledge of physics and chemistry gained over the course of six industrial revolutions, we have developed the ability to manipulate and engineer matter, and by doing so we have rediscovered and harnessed the results of six similar revolutions that occurred during billions of years of biological evolution.

Using nanobiotechnology, we stand at the door of manipulating genomes in a way that reflects the progress of evolutionary history: starting with the simplest organisms and ending, most portentously, by being able to alter our own genetic makeup. Synthetic genomics has the potential to recapitulate the course of natural genomic evolution, with the difference that the course of synthetic genomics will be under our own conscious deliberation and control instead of being directed by the blind and opportunistic processes of natural selection.

We are already remaking ourselves and our world, retracing the steps of the original synthesis—redesigning, recoding, and reinventing nature itself in the process.

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* Bacteria called *Clostridium perfringens* and *Vibrio natriegens* seem to be the world's fastest doublers, reproducing in seven to ten minutes respectively.

CHAPTER 1

-3,800 MYR, LATE HADEAN
At the Inorganic/Organic Interface

What follows is the greatest story ever.

It's the story of a once invisible being, nameless for eons, now called "the genome." Its being—its existence across time, its depth and complexity as a natural artifact, and the vast abundance and variety of its manifestations—is the story. It is ancient and modern, older than our oldest ancestor and yet fresher than a newborn baby. It has covered our planet with its descendants, now over a billion times a billion times a billion copies (10^{27}).

The tale of the genome involves more sex than the most pornographic novel imaginable. The narrative is replete with incredible action scenes, countless life-and-death struggles, wild improbabilities that turn out to be true, and overwhelming successes in the face of staggering odds. It is a story about families and universal truths. In the retelling, it becomes, in part, your own personal story. The tale reveals a vibrant past and may lead us to a better future. As the ultimate self-help manual, it offers better health and longer life, along with "descendants as numerous as the stars in the sky and as the sand on the seashore" (as in the Judeo-Christian-Islamic tradition), or "as numerous as the sands on the Ganges" (in Buddhism).

As befits the greatest story ever, this is a multiplex tale, enacted and told in a spiral of understanding. Through its abundance, fidelity, and diversity, the genome adapted to the physical world, solving a small number of basic problems repeatedly, passing on the answers, and occasionally even rediscovering solutions once lost. We see these problems solved in the first instance biologically, by the process of evolution. Nature turned inorganic materials into organic substances. Natural organisms read and interpreted genomes. And natural organisms have created huge amounts of genetic diversity. That network of natural interactions comprises our first tale.

It begins long ago, in the Hadean era.

Can Organic Arise from Inorganic? Selection Among Atoms and Molecules

The Hadean geologic era lived up to the image of an underworld inhabited by the ancient Greek god Hades—lifeless and full of hot lava—3.8 billion years ago. If a living cell were unfortunate enough to travel back through time to the Hadean landscape, it would be cooked: all water vaporized and its precious complexity of living stuff dry-roasted and then mineralized, turned from delicate, filmy proteins into charcoal (graphite), water vapor, and other waste products.

Before this, all the way back to the big bang, the universe was made up almost entirely of hydrogen nuclei, the simplest of all elements, consisting of just one proton. These protons would collide and fuse together to form helium nuclei (2 protons). Inside stars these helium nuclei would in turn fuse to form carbon (6 protons). Carbon nuclei would

then enter a cycle (the carbon-nitrogen cycle), taking in hydrogen, and by adding nitrogen (7) and oxygen (8) intermediates, would catalyze the formation of yet more helium. The new helium would, as before, make more carbon. The net outcome of all this is that in hot stars carbon catalyzes the formation of copies of itself. (By "catalyze," I mean causing or accelerating a reaction without the catalyst itself undergoing a permanent change.)

These thermonuclear transformations, which occur at Hades-plus temperatures within stars, are accompanied by the release of enormous amounts of energy in the form of radioactive particles such as gamma ray photons, positrons, and neutrinos. (And also of course by the heat and light that drive life on this planet.)

The processes that make up the carbon-nitrogen cycle can be thought of as a form of natural selection for favorable reactions and stable elemental forms (atoms and their isotopes). This seems analogous to the mutation and selection of living species, and still later the mutation and selection of synthetic organisms. Today those five (hydrogen, helium, carbon, nitrogen, and oxygen) of the eighty stable elements are the most abundant in the universe. These processes selectively skipped over weakly represented lithium (3), beryllium (4), and boron (5).

A list of such atomic elements (substances that chemically cannot be broken down further) is a prerequisite for understanding the next level of selection complexity—the combination of those basic atoms into the compounds (molecules) of nature. Antoine Lavoisier wrote the first comprehensive list of the elements in the first modern chemistry text, *Traité élémentaire de chimie*, in 1789. He listed thirty-one in all, together with light and "caloric" (heat), making up a total of thirty-three "simple substances belonging to all the kingdoms of nature, which may be considered the elements of bodies." As Lavoisier presented them:

LIGHT	SULFUR (S)	ANTIMONY (SB)	MERCURY (HG)	CALCIUM (CA)
CALORIC	PHOSPHORUS (P)	ARSENIC (AS)	MOLYBDENUM (MO)	MAGNESIUM (MG)
OXYGEN (O)	CARBON (C)	BISMUTH (BI)	NICKEL (NI)	BARIUM (BA)
NITROGEN (N)	CHLORINE (CL)	COBALT (CO)	PLATINUM (PT)	ALUMINUM (AL)
HYDROGEN (H)	FLUORINE (F)	COPPER (CU)	SILVER (AG)	SILICON (SI)
	BORON (B)	GOLD (AU)	TIN (SN)	
		IRON (FE)	TUNGSTEN (W)	
		LEAD (PB)	ZINC (ZN)	
		MANGANESE (MN)		

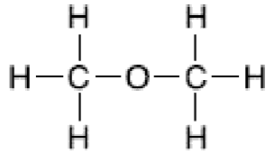
Each element in the table above is followed by the abbreviation that is commonly used in most branches of science, and even within the general culture—for example, H_2O (water), NaCl (salt), and CO_2 (carbon dioxide). Jöns Jakob Berzelius, who developed an interest in chemistry in medical school, introduced these symbols in 1813. By 1818 he had measured the masses of forty-five of the eighty stable elements. As we will see in Chapter 3, as few as six elements may be sufficient to create the major molecules of life: S, C, H, P, O, N (sulfur, carbon, hydrogen, phosphorus, oxygen, and nitrogen—pronounced "spawn"—shaded gray in the table above). These constitute the most abundant elements in living systems; also needed are metal ions such as magnesium (Mg) that are involved in key reactions of these compounds.

These elements chemically combined with one another to form molecules, such as water, as the newly formed earth cooled. How did life arise from nonlife? To understand this, we need to explore the universe of simple, nonliving chemicals. As far as we know, the physical and chemical properties of the elements are set largely by particles in the nucleus (as well as by those in the surrounding electron cloud), and not by the specific arrangement of those particles. For example, it matters only that there are six protons in carbon; the exact structural relationships among the protons are irrelevant. Those six protons, irrespective of how they are arranged in the nucleus, attract and retain an equivalent number of electrons in the surrounding electron cloud.

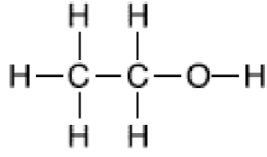
In molecules, by contrast, the physical arrangement of the component atoms is crucial.

For example, a molecule of water, H_2O, is not just ten protons and ten electrons packed together randomly in a jumble. The order of the atoms and their shape matters. Water is not H-H-O but rather H-O-H, meaning that each hydrogen atom can only bind to the oxygen atom, and not to two atoms. Molecules are like intimate social networks. Some atoms, such as hydrogen, tend to make single bonds with only one other atom. Oxygen makes two bonds, nitrogen three, while an atom of carbon can bond with four other atoms. So, water has each hydrogen bonding with one atom, oxygen, and its oxygen bonding with two atoms.

Let's now replace each hydrogen in water with a carbon (keeping each carbon happy with its own three hydrogens): this will give us dimethyl ether, CH_3-O-CH_3. So let's check the bonds. The oxygen still has two single bonds—one to each carbon—and each carbon has four single bonds, three to hydrogens and one to the central oxygen.



Now we can illustrate the importance of spatial arrangement. If we keep all nine component atoms but rearrange them slightly, say to CH_3CH_2OH, we get a radically different set of physical and chemical properties in a molecule called ethanol.



What a difference that simple rearrangement makes! Dimethyl ether boils at -24 degrees C while ethanol boils at +78 degrees C. Many people like to drink ethanol (typically 8 to 15 percent in water), but you would not want to drink dimethyl ether. These rearranged molecules are called isomers of each other (Greek for "the same parts"). Ethanol is an isomer of dimethyl ether: each molecule has two carbons, six hydrogens, and one oxygen, but differently arranged.

Berzelius came up with the concepts and terms for catalysis, polymer, and isomer, among others. He also provided experimental evidence for the law of definite proportions (first stated by the French chemist Joseph Proust), which holds that the proportions of the elements in a compound are always the same, no matter how the compound is made. Even though we have been introducing these ideas by appealing to the simple bonding of discrete atoms, Berzelius discovered them by doing two thousand analyses over the course of a decade, purifying and weighing chemicals and their reaction products. He noticed that the ratios were reproducible and generally came in values that were expressible in whole integers. Berzelius was also the first to recognize the difference between organic compounds that were derived only from living matter, and all other chemicals, which he lumped together as "inorganic." This distinction contributed greatly to our understanding of life and set the stage for inquiries into vitalism, the theory that life and its processes are not reducible to the laws of physics and chemistry. Berzelius believed that something kept living matter distinct from nonliving matter. But work done in four areas—the synthesis of urea, the investigation of mirror molecules, the investigation of polymers (especially of the DNA/RNA polymers), and the self-reproduction of molecules—argues to the contrary.

Berzelius's protégé Friedrich Wöhler also came to chemistry through the study of medicine. In 1828 Wöhler (accidentally) became the first person to synthesize an organic

compound, urea, from an inorganic substance, ammonium cyanate. The reaction in question is $NH_3HNCO \rightarrow NH_2CONH_2$. This is a rearrangement of atoms similar to that of the isomers mentioned above. But at the time it was more mysterious, in part because the description of chemicals as precise arrangements of atoms was just becoming evident from experiments. Second, urea was thought to come only from the urine of certain vertebrates as well as, less obviously at the time, other species. Ammonium and cyanate were considered to be inorganic components of minerals.

Wöhler's synthesis of urea was arguably the first great challenge to vitalism. Since then, scientists have tried to make ever more complex organic living systems from inorganic or otherwise simple nonliving atoms and molecules. With hindsight, urea was a very simple case (consisting of just eight atoms of carbon, hydrogen, oxygen, and nitrogen) and was thus poised for success in this first of five grand challenges to vitalism —all of which reflect milestones in practical synthetic biology as well.

The second challenge to vitalism concerns the phenomenon of the handedness of molecules—one of the distinguishing features of living systems. The challenge is to determine whether natural single-handedness can arise spontaneously or be reversed, and if so, what the consequences would be.

The chemistry of life is based on polymers made by linking monomer molecules together in long linear sequences, just as written texts are made of linear sequences of letters. These two terms share the common root "mer," from the ancient Greek *meros* for "part." A monomer, accordingly, is a single molecule (one part), whereas a polymer (many parts) is a molecular structure composed of many similar molecular units bonded together. Amino acids are monomers whereas combinations of them are polypeptides (a.k.a. proteins), which are polymers. The large molecules known as RNA and DNA are also polymers—polynucleotides—consisting of many simple molecular subunits known as nucleotides. Those three types of polymers can bind and catalyze the formation of other polymers as well as the metabolism of the basic components of living things. A single typo in a biopolymer sequence could make the polymer nonfunctional and nonliving. So the *third challenge to vitalism is to find out whether those long, precise sequences could arise spontaneously and possess the functions of life such as catalysis*. Can new kinds of life exist that have no ties to ancient life—a truly artificial or synthetic life form?

The fourth challenge is determining whether a fully synthetic chemical network could make a copy of itself and evolve (i.e., change with time) and in so doing, prolong its own survival. And *the fifth challenge is whether consciousness (or a mind) can arise synthetically*. This will be addressed in the Epilogue.

Is Biological Handedness Special? What Are the Consequences of Reversing It?

This section will consider the second challenge to vitalism: biomolecular handedness. There are six compelling reasons to care about handedness.

First, when we inspect meteorites and other matter that has fallen to the earth from space, we look for an excess of molecules of the same handedness (one "enantiomer," meaning one of a pair of molecules that are mirror images of each other). In space there are more molecules of one specific handedness than of the other. Does this mean that life arose far away and landed here, or rather that one hand is more likely to spontaneously arise or survive? The answer to this question has profound implications for our place in the universe.

Second, the two different hands have different pharmacological effects. The drug thalidomide was used in Europe between 1957 and 1961 to treat morning sickness in pregnant women. Thalidomide was made chemically and not biologically and hence both hands were made in relatively equal amounts. It turns out that one hand cures the morning sickness while the other causes severe limb malformations in the developing fetus (a result described by the BBC as "one of the biggest medical tragedies of modern times").

Third, chemicals whose molecules exist in only one spatial arrangement tend to be

more economically valuable than those that are mixtures of molecules having a given arrangement together with those of their mirror images. The “unnatural” versions are more expensive (1,400-fold more for the amino acid isoleucine).

Fourth, the oceans contain a large mass of carbon trapped in the form of recalcitrant dissolved organic matter (the ominous sounding RDOM), much of which consists of mirror-image forms of easily recycled (nonre-calcitrant) matter. The handedness of these trapped carbon molecules causes them to persist in the oceans for millennia.

Fifth, the ability to reverse the handedness of useful polymers, such as cellulose, wool, and silk, could retard decay. Biodegradable plastics may come to be seen as a mixed blessing. The usual route of biodegradation is through release of carbon dioxide, which is currently an unwelcome output. Also, the energy normally expended in recycling or replacing degraded polymer products might be saved in some cases.

Sixth, at the extreme, a mirror cell or a mirror organism (composed of chemicals of reversed handedness) might be resistant to all or nearly all parasites and predators, a tremendously valuable result.

Since biomolecular handedness is so important, what is it? The basic idea is conveyed by the fact that our right and left hands are mirror images of each other and are not related by simple rotations. If we take a sculpture of a right hand and press it into a soft mold, we will discover that we cannot fit our left hand into the mold (Figure 1.1). However, if we fill that mold with plaster, the resulting new copies are considered complementary and are of the same handedness as the originals.

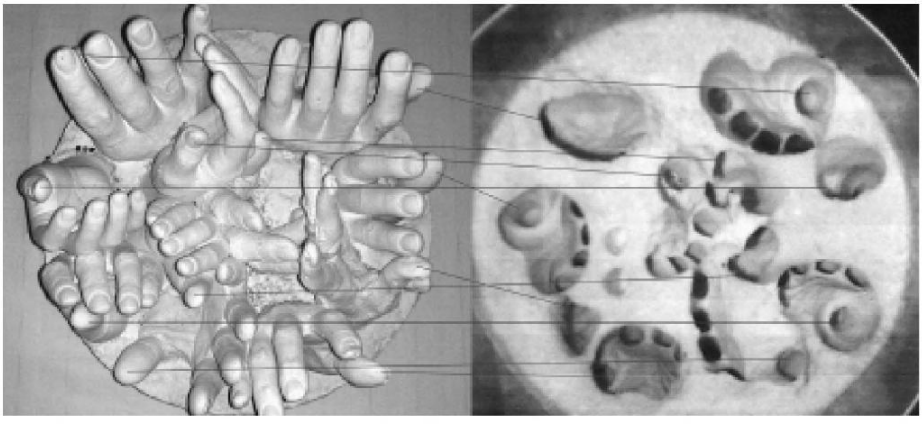


Figure 1.1 A sculpture on the left and corresponding negative mold—illustrating handedness and complementary shapes.

This same phenomenon exists on the molecular level. For example, there are two ways to arrange the four atoms that can bond to a carbon atom, and each will be a mirror image of the other. Furthermore, each will have predictably similar properties.

This left-right feature is also known as chirality, from the Greek (χείρ) for “hand.” Even scientists who don’t think about mirror worlds initially show great confusion as to whether the properties of mirror versions of molecules, cells, and bodies can be accurately predicted based on the properties of their nonmirror versions. Consider this. If you build a replica of an old-fashioned clock by only looking at its reflection, the copy will predictably tell time, but the numerals will be mirror images of the originals and the hands will rotate counterclockwise. These outcomes are precisely as anticipated.

Here’s a simple demonstration that relates the hands and clock examples to molecules. Start with a central cantaloupe ball, and use toothpicks to successively place around it, in a clockwise order, a raisin, a piece of coconut, and a piece of nectarine all flat on the table. Then make another such structure using the same pieces of fruit but placing them

counterclockwise. You can flip one over so that the two structures match, but if you add a bit of honeydew above and attached to the central cantaloupe, then no matter how you orient the structures you can still tell which was clockwise originally and which wasn't. If you place them in front of a mirror you can see that they are each other's mirror image. Now let's replace fruit with atoms: H, CO, R, N. This is the general structure of an amino acid. The NH_2 is the amine and COOH is the acid. R refers to a "radical" (a group of atoms that behave as a unit) that varies with amino acid type.

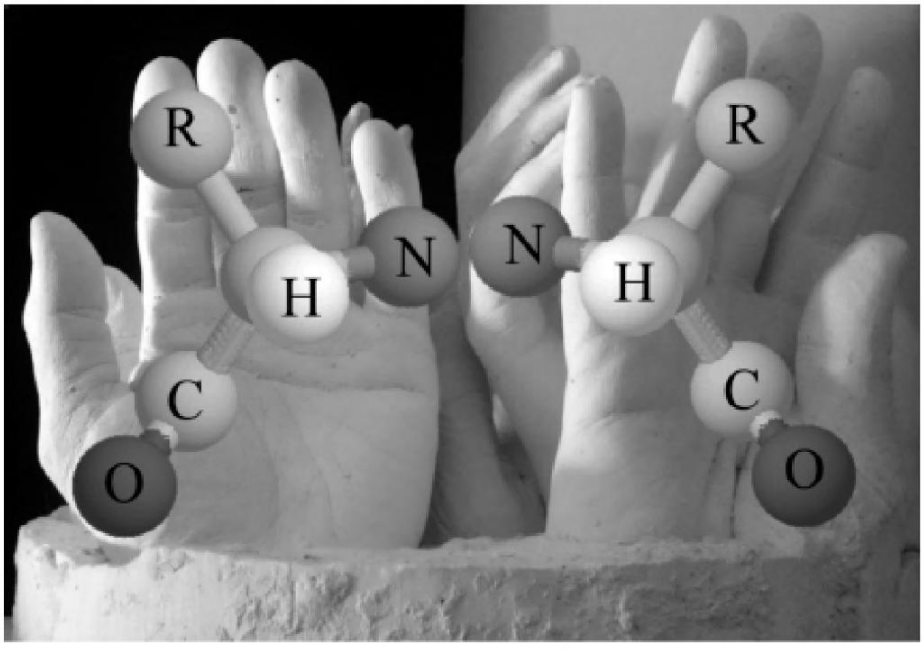


Figure 1.2 Another example of handedness uses a ball and stick model of a carbon atom with its four bonds (to H, CO, R, N groups).

Amino acids have a known handedness. You can impress your friends by your ability to identify the natural form. In nature, for reasons still unknown, almost all biomolecules vastly prefer one of the two hands (amino acids and proteins being designated as left-handed). Life itself, in a way, is fundamentally single-handed. Here is a procedure for telling whether a human hand, or a molecule, is right- or left-handed. Looking at your left hand palm up as in Figure 1.2, go from thumb to index finger to pinkie, the direction is clockwise, which indicates left-handedness. Performing the same observation on the right hand gives a counterclockwise direction, indicating right-handedness.

Now let's do the same for molecules. When looking down the bond from the hydrogen (H) to the central carbon, if the other groups going clockwise are CO, R, N, as on the left of Figure 1.2, then the configuration is normally seen in natural proteins (sometime called *levo* or L, for left-handed). On the right is the mirror version (*dexter*, Latin for "right," or D). The R (radical) group distinguishes the twenty (or so) types of amino acids, each with its own personality (and its own single-letter code). Some are electrically negative while others are positive. Some are greasy and fear water (or hydrophobic), while others love water (hydrophilic). Glycine is the only amino acid that is its own mirror image, since its two hydrogen atoms are normally indistinguishable. Just to keep us on our toes, natural nucleic acids (RNA and DNA) were long ago designated D and their mirror forms L.

By now you may be wondering about the cash value of this talk about handedness. Just as there can be mirror molecules, there can also be mirror life. Mirror life would be the result of changing the handedness of an entire organism and all of its components, so

that you have a mirror image of everything from the macro level all the way down to the atomic level. While mirror life may *look* identical to current life, it would be radically different in terms of its resistance to natural viruses and other pathogens. Mirror life forms would be immune to viruses and other pathogens, the reason being that the molecular interactions of life are exquisitely sensitive to the mirror arrangement of their component atoms and molecules. Normal viruses would not recognize a mirror organism as a genuine life form whose cells it could invade and infect. Such multivirus resistance would be an incredible boon to humanity. But it would come at a steep price because mirror life would be unable to digest foods by means of normal enzymes, which would mean that we would need to develop, cultivate, and mass-produce a whole range of mirror foodstuffs. (Although biohackers could in principle synthesize mirror viruses and other pathogens, mirror humans would still be resistant to natural pathogens—and even to genetically engineered nonmirror superpathogens.)

The prospect of mirror humans raises unusual and startling possibilities. Supposing that there will be a transition to a mirror version of human beings at some point in the distant future, the changeover would be gradual, with a substantial interregnum period when two types of human beings would exist: natural humans composed of natural-handed molecules, and mirror humans made up of mirror versions of them. In this situation, it's almost as if two separate species of humans existed simultaneously. Or we might see an equilibrium between the two types if mirror pathogens arose.

These mirror humans should have an unusual smell. Members of the two versions could marry, but producing children would require what today would be considered extraordinary efforts. However, by the time we can make mirror humans, making designer (or random) children of either mirror type will not seem as challenging as it does today. We might even be able to make mirror identical twins or bodies that are mixtures of both types of cells.

Finally, creating a race of mirror humans is not without risks. Although new mirror molecules interact with mirror versions of existing molecules in predictable ways, how they interact with biomolecules in general is unpredictable. However, they are no more unpredictable than any newly synthesized drug, chemical, or material; nevertheless, careful screening of mirror molecules by computational methods or by actual experiment will be necessary to ensure safety.

Louis Pasteur had the first inklings into what natural chemical chirality is all about. He acquired this understanding by performing what the magazine *Chemical and Engineering News* once referred to as the "most beautiful chemistry experiment in history."

Pasteur's seventy-two years on earth are remembered mainly for his contributions to microbiology—especially for inventing pasteurization, discovering the "Pasteur effect" (the anaerobic growth of organisms), developing the first vaccines for rabies and anthrax, and contributing to the understanding of fermentation, as well as for his clever experiments in support of the germ theory of disease. Nevertheless, his earliest and equally great achievements came from his work as a crystallographer. In 1848, the seventy-three-year-old French physicist Jean Baptiste Biot sponsored the twenty-five-year-old Louis Pasteur in his first experiments at the elite college École Normale Supérieure in Paris.

This is a story about tartar, a chemical extracted from grapes. The modern chemical term "racemic" (meaning a mixture of the two hands) comes from the Latin *racemus* for "cluster of grapes." In 1838 Biot found that tartaric acid, unlike its isomer, racemic acid, was optically active, meaning that it rotated the plane of a beam of polarized light. Both isomers are found in wine, the latter in sediments or by heating tartaric acid. These two acids were one of the first examples of an isomer pair and they turned out to be unusual in that almost all of their physical and chemical properties were identical except for their solubility and their ability to rotate polarized light.

amounts, they would have annihilated one another. There is no consensus yet on the explanation of this asymmetry. Similarly, if there had been equal amounts of left- and right-handed molecules, life might not exist in the universe—at least not life as we know it. In any case, once we get replication, then we can expect to see, more and more frequently, small random events that can grow exponentially into interesting structures before any competing chemistry can take hold.

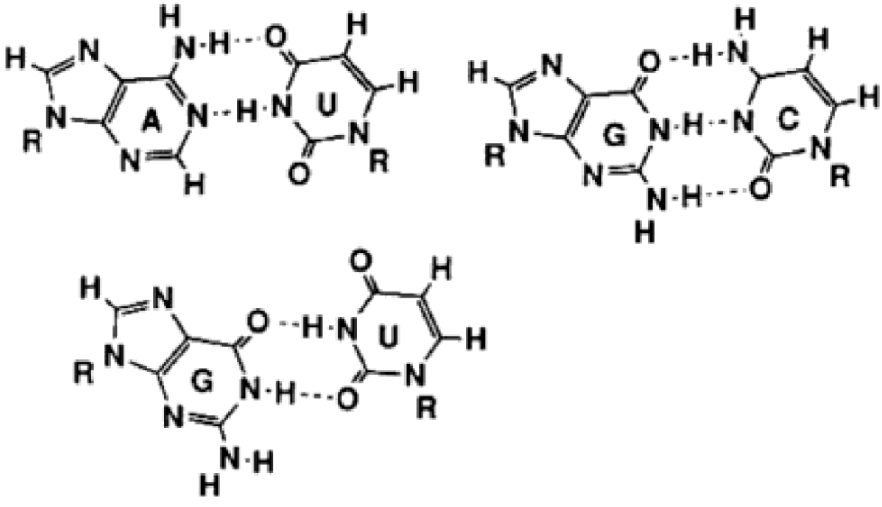


Figure 1.4 Complementary shapes of RNA base pairs. Shown at the top are the two dominant base pairs (AU and GC); just below them is an example (GU) of the other eight possible (very weak) base pairs. What seems like a subtle difference in geometry between the AU pair and the GU mispair makes for a huge difference in the context of a stack of these flat base pairs in the double helix. (The R represents the ribose sugar to which all four bases bind with very similar geometry.) The dotted lines are bonds mediated by hydrogen that are about one hundred times weaker than the covalent bonds (solid lines) in their optimal configuration.

Evolution happens not only in nature but also in the laboratory, where the key processes of mutation and selection operate on inanimate molecules and structures made up of them. Even creationists can see how small changes, when made repeatedly over long stretches of time, can add up to enormous effects that confer substantial selective advantages on a given organism. What is more remarkable is how new kinds of functionality and shape can emerge out of totally random collections of RNA rather than as mere variations on something already optimized and working. This process of emergence has major implications for how quickly new genes and genomes could have arisen in the past, as well as for the design of medical and industrial materials in the near future. Totally random libraries of RNA can be subjected to powerful selection pressures that favor rare molecules capable of valuable binding or catalysis functions. We can generate an incredible number of different RNA structures in a volume equivalent to that of a small cell. If any of these RNAs has any activity for preferentially cutting and/or joining, then the whole set of RNA sequences could churn and self-modify until stable self-replicating molecules arise and persist.

So, the answer to the question posed earlier—Can a synthetic chemical copy itself and evolve without help from living systems?—is a resounding yes. Here is an example of such evolution in the lab. A molecule of theophylline (which is used as a drug to treat asthma and other lung diseases) can form part of a fifty-five-nucleotide-long stretch of RNA that can have two different morphologies and two different functional states depending on the concentration of theophylline. It is easy to imagine that this molecule could start with either state as its “only” shape and function and could change to the bi-stable shape with as little as the mutation of a single nucleotide. Then after some other molecule adapts to the bi-stable state, another point mutation locks it into one state or the

other, permanently.

The moral of the story is that shape and function can be altered radically with just a few changes that nevertheless yield a selective advantage at each separate stage. This capacity will be very handy in the future of lab-evolved designs.

The Future Interface of Inorganic and Organic Worlds

We have been focusing on inorganic and organic chemistry. In colloquial usage the term "organic" is attended by a certain halo effect that, upon analysis, it doesn't deserve. When we buy organic produce, we are supporting the idea of feeding crops the essential elements nitrogen and phosphorus that are derived only from animal excrement rather than from conventional mineral fertilizers like ammonium phosphate as churned out by the chemical industry. Does this sound like a latter-day vestige of vitalism? These organic fertilizers obviously bear a public health risk in the form of fecal pathogens such as *E. coli* 0157:H7, *Cryptosporidium*, and *Giardia*. Both methods of fertilization, if used to excess or done poorly, carry a risk of run-off into streams and ponds resulting in fish kills.

Another inorganic/organic dualism can be seen at the interface between life and machines. I/O means not only the intimate dance of inorganic/organic, but also input/output. Today scientists are recapitulating what we might call the first inorganic/organic transition that occurred eons ago. We take simple molecules and form them into linear polymers that are the building blocks of both natural and synthetic structures. We increasingly want to see input/output between inorganic electronics and organic DNA. On the input side of I/O, megapixel CCD (charge-coupled device) and CMOS (complementary metal oxide semiconductor) electronic cameras can be used to record spatially patterned light, such as bioluminescence or fluorescence, to inorganic (i.e., silicon-based) computers. This would allow us to read genomes speedily, whether for diagnostic testing or environmental monitoring. Coupling these inorganic/organic, input/output features together permits us to design, synthesize, and assess the quality of large collections of DNA and anything that they encode.

Back in the early stone age of DNA engineering (circa 1967–1990) we made DNA in solution and had to purify very short intermediate products. The low yields for each step, multiplied by the short lengths per step, made DNA synthesis a challenging, tedious enterprise. Nowadays we can literally "print" arrays of DNA by machine. This is a really big deal. To see why, let's explore analogies with other types of printing.

Today we use spatially patterned light and optics or ink-jet printers to print photographs on paper, which are two-dimensional artifacts. But it is possible for those same ink-jet printers to "print" (i.e., to construct, layer by layer) three-dimensional objects. Ink-jet systems can hold many colors and activate many jets in parallel. If the ink consists of colored minerals or glue, then we can deposit (or "print") one layer on top of a second layer (typically 0.1 mm per layer), and then repeat this process successively to create three-dimensional rapid prototypes of artifacts in plastic or plaster.

We can use similar approaches of spatially patterned light or ink jets to build up long chains of DNA called oligonucleotides, or "oligos" (from the Greek *oligos*, for small), up to 300 nucleotides in length. Typically each layer is one nucleotide (= 0.4 nm) thick, with the four ink-jet "colors" (A, C, G, and T) used per layer. By this method we can make millions of different patches of DNA on a 3- by 1-inch glass slide or portion of a larger silicon wafer.

In 1980 commercial DNA synthesis services were available, at the going rate of $6,000 for a small amount of product, only about ten nucleotides long. They were used either to find valuable genes in cellular RNA or to synthesize them. By 2010 we could make a million 60-nucleotide oligos for $500. Just as the global appetite for reading DNA seems insatiable—growing a million-fold in six years and still increasing—the appetite for DNA synthesis, or "writing," will probably grow similarly and go in many unexpected directions. Since DNA in cells is very long-lived (billions of years), we might want to preserve the whole Internet in the form of DNA molecules. This would be the ultimate backup, made possible by converting the Internet's 0s and 1s to the DNA molecule's As,

Cs, Gs, and Ts, and synthesizing the molecules accordingly. The Internet Archive contains 3 petabytes (10^{15}) of data, and is expanding at the rate of 1 petabyte per year. This granddaddy of all backup copies would cost $25 billion, an amount that is not out of the question, but bringing that cost down by three to six factors of ten would be desirable. Because of its very small size, launching copies into space and icy moon polar craters could be very inexpensive.

Today, oligonucleotide chips are becoming the lifeblood of synthetic biology. However, spatially patterned light and ink-jet printers can be used to make objects as complex as patterned cells. Various options exist: (1) the cells themselves can be shot directly from ink jets, (2) scaffolding proteins can be deposited in such a manner that the cells self-assemble onto those proteins, or (3) the cells can be assembled onto photo-reactive scaffolding and then selectively stabilized or released by light. These and other methods hold the potential of making synthetic and even personalized tissues and organs suitable for testing pharmaceuticals—and ultimately for printing copies of whole organisms.

As we go forward we will be seeing more hybrid inorganic/organic systems. Our children already inherit our mechanically augmented biology, in the form of cars, smart-phones, hearing aids, pacemakers, and so on, and these devices have become increasingly integrated into our daily lives; indeed, many people would find it hard to live without them. Since the 1980s we have added recombinant DNA-based parts to our bodies in the form of insulin, erythropoietin, monoclonal antibodies, and other medically useful substances. The addition of complex synthetic biological systems to this mix will ultimately blur the distinction between life and nonlife.