WHAT IS LIFE?

INVESTIGATING THE NATURE OF LIFE IN THE AGE OF SYNTHETIC BIOLOGY

ED REGIS

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Investigating the Nature of Life
in the Age of Synthetic Biology

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Published by Arrangement with Farrar, Straus and Giroux, LLC

First published as an Oxford University Press paperback, 2009
198 Madison Avenue, New York, NY 10016

www.oup.com

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Library of Congress Cataloging-in-Publication Data
Regis, Edward, 1944–
What is life? : investigating the nature of life in the age of
synthetic biology / Ed Regis.
p. cm.
Originally published : New York : Farrar, Straus and Giroux, c2008.
Includes bibliographical references and index.
ISBN 978-0-19-538341-6 (pbk.)
1. Life (Biology)—Philosophy. 2. Vital force. I. Title.
QH501.R434 2009 570.1—dc22 2009003487

1 3 5 7 9 8 6 4 2

Printed in United States of America
on acid free paper

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Prologue

The Second Creation

IN THE SUMMER OF 2002, in northern Italy, a group of three scientists and a philosopher agreed to create a new form of life. The four were old friends, buddies, and during the course of an annual reunion they realized that at this precise stage of their respective careers each of them wanted to be working on some important, ambitious, and challenging new project.

So they decided they would create life. Not a simulation of life. Not an imitation of life. Not fake life. Rather, real life, a genuinely new living entity, albeit one not based on biology and not made out of the customary biological ingredients: no DNA, no conventional biomolecules, no cell membrane of the ordinary type, no nucleus, no mitochondria, no endoplasmic reticulum or any of the other innumerable vital trappings of normal, orthodox biological cells.

None of the group members was sure what the exact definition of life was, if indeed there was one, although metabolism, self-reproduction, and the ability to evolve seemed to be essential defining criteria of any entity that was to be classified as alive. For the time being, however, the obstacles to creating new life were not definitional, but rather scientific and technical.

For one thing, creating life from scratch had never been done before—at least not by scientists. Life on earth had a genesis, that was for sure, but what the exact order of events had been that led to the origin of life was, to put it mildly, an unsettled question in biology.

The prospect of creating new life also raised some entirely novel technical questions. What kind of food would an artificial organism eat? Would new life come into existence all at once, like a bolt of lightning, or gradually, in stages? Would it need some sort of chemical weaning process, a form of artificial life support?

Where would the money come from? What lab or labs would be involved, and in what countries? Could a passably blasphemous project such as this be attempted in America, many of whose citizens were unduly alarmed even by the idea of genetically modified foods? What were the potential risks involved? Was a population of synthetic living entities going to be dangerous, a threat to civilization?

The project would be no trivial undertaking, but that only added to its appeal: there was no glory in doing something easy. Creating life would be, as Robert Oppenheimer had said of building the atomic bomb, "technically sweet," a goal scientifically so tempting as to be almost irresistible.

The group had no guarantee of success, but they had sev-

eral things going for them, the most important of which was that life had already emerged once: in other words, they had a proof of concept. Second, modern science had an extremely good understanding of how life worked, right down to the smallest molecular details. And so conventional life, its general architecture, mechanisms, and arrays of vital processes, could be used as models for new life-forms, examples of which the researchers would produce in different media and with different raw materials.

Third, science had ascertained with the utmost precision how certain chemicals reacted with others and how a given type of molecule could hold information and control the activities of other molecules, or groups of them—all of which meant that the current level of chemical knowledge was potentially up to the task of building a living, metabolizing synthetic cell. Fourth, machine technology had reached the point where miracles of manipulation could be performed by devices that worked at the very finest physical scales, which was a capacity the scientists could exploit in the creation of their microscopic artificial organisms. Fifth and last, there was the computer. By this point, practically any envisioned process, entity, or anything else could be simulated beforehand, in arbitrary detail, and repeated as many times as you wanted—previewed, reviewed, revised, rewound, run backward—all to serve as a guide to the physical realization of the phenomenon in question. And so it should be possible to simulate, well ahead of building it, the full developmental path and the complete inner workings of an artificial living cell.

In the progress of science and technology there has often been a distinct, ripe psychological moment at which a given

revolutionary undertaking that was previously impossible becomes suddenly doable—a project uniquely suited to the times. And at this precise moment in history, the time was right for creating life. The scientists might fail in their attempt, but surely others would succeed, for there was unlikely to be any insuperable barrier to the ultimate realization of their goal.

In any case, success, or even failure, would raise an additional bunch of questions, ones that reached far beyond the narrowly technical issues. What were the stakes here, scientific, moral, political, legal? The problems started with the perennial and trite layman's taunt, the claim that creating life was "playing God." That comment had been spat out as an insult aimed at nearly every radical scientific advance, from splitting the atom to birth control to genetic engineering, organ transplants, and whatever else. Still, the question would have to be faced.

Behind the layman's taunt, however, was a cluster of legitimate concerns. Would the attempt to create new life venture into forbidden territory, sacred, untouchable realms into which mere mortals shouldn't even think of entering? Had science, in other words, finally reached its proper stopping point?

What light would a new form of living matter shed, if any, on the "meaning" of life—whatever was to be understood by that mystifying phrase? What would it say about the worth and uniqueness of human life, or of life on earth, human as well as animal? Would human life, or any form of life, have less value if we could create new life-forms at will, like works of art? Were animal rights activists going to claim that these

new life-forms had rights, too? Would new life be more like medicine . . . or more like poison?

For that matter, a collection of hot-button, lightning-rod issues swarmed around the concept of life like bees around a hive: the problem of abortion, for example, which rested in part on the question of when human life began. At the other limit of human existence, the issue of euthanasia, the use of heroic measures to artificially prolong life, and when to withdraw them, were bound up with the determination of when life, in any meaningful sense, ended. There were the further problems of our moral obligations to other species, especially to those endangered species, such as insects, that might be considered "lower" forms of life; whether the harvesting of embryonic stem cells constituted the taking of a human life; whether an advanced form of artificial intelligence, if and when it ever existed, would itself be a new kind of life-form; and so on.

The scientists involved in creating new life were not motivated by a desire to answer such questions—nor had they any special competence for doing so. Science, after all, was concerned with what is, not with what ought or ought not to be. The most profound and provoking question raised by the effort to build an artificial living cell, therefore, was exactly the one that lurked as an unseen presence beneath all the rest: the age-old riddle, What is life?

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One

Birth of a Cell

MAY 2005. In a new industrial park at Porto Marghera, some four miles across the lagoon from Venice, an American physicist by the name of Norman Packard is staring at the enormous 30-inch-wide display screen of a Macintosh G5 computer. Floating around against a dark background is a dense assortment of red, green, and blue dots.

"Blue is water, the greens are hydrophobic molecules, which means they don't like water, and the reds are hydrophilic molecules, which do," Packard says.

The simulation begins with the dots spread out evenly across the screen in a relatively homogeneous mix. But then in the incremental time-steps of the particle dynamics program, a pattern emerges. The greens move toward one another and then converge and clump together, forming a spherical structure. The reds, meanwhile, follow the greens and arrange

themselves on the outside of the mass, as if to protect it from intrusion. The result is a vesicle, a tiny bilayered fluid-filled sac. The vesicle has formed itself spontaneously, the result of a self-assembly process driven by Brownian motion (the random thermal movement of molecules in a fluid medium) and by various chemical reactions.

"We believe that this combination of chemical reactions and self-assembly is one of the crucial combinations that we need to understand to make these artificial cells," Packard says.

Artificial cells? Venice? A city of more than a hundred churches, miles of canals, and innumerable ancient palazzi, all of them suspended in time, a place where nothing fundamentally new has happened for hundreds of years? Somehow the location is strangely fitting. In its heyday, Venice was a world-class power and trading center as well as a realm of considerable intellectual freedom. The city was now and always had been home to a variety of creative spirits: composers, artists, and scientists, including Galileo. And its labyrinthine streets and alleys were bathed in the green waters of the Venetian lagoon—water just coincidentally being the medium in which, according to most theories, earthly life originally began. So why should it not begin again, here?

Norman Packard, for one, finds no incongruity in the prospect. Packard is the chairman, CEO, and scientific head of ProtoLife s.r.l., a Venetian start-up company located in Parco Vega, a technology park the regional government had created on the grounds of an old chemical factory.

"The city of Venice, but even more generally the region of Veneto, wants to diversify its portfolio of activities," Packard said. "Venice has this very strong component of tourism that

dominates its economy in many ways, and so it's trying to create some economic diversity that can give a certain kind of life to the city, not related to tourism."

ProtoLife's business plan is founded on an attempt to start life over, to begin from the beginning. It's not their intention to redo Genesis, outdo Frankenstein, or to blaze a path of glory through one of the final frontiers of applied science—although, if they're successful, Packard and his crew will end up doing all those things. The company's motivation is far more prosaic, practical, and commercial: to create artificial cells. Made from scratch and called "protocells," they will be programmed to carry out useful tasks such as synthesizing vaccines and drugs, cleaning up toxic waste, scavenging excess CO_2 from the atmosphere, and other such miracles, and earning the company a tidy profit in the process.

After watching his simulation run a few more times—"We've done between six and seven thousand runs so far," he says—Packard walks down a polished green marble hallway, turns right, unlocks a door, and enters the company's lab suite. This is the domain of ProtoLife's chief chemist, Martin Hanczyc, a postdoc Packard recently hired away from Jack Szostak's competing artificial cell project at Harvard. In fact, ProtoLife is only one of a half dozen or so scientific efforts bent on creating new life: in addition to the ProtoLife and Harvard projects, there are others at Rockefeller University in New York, the University of Nottingham in England, and the University of Osaka in Japan, among other places. All too obviously, creating life is an undertaking whose time has come.

Hanczyc's laboratory at ProtoLife boasts a full supply of chemical apparatus: the usual lab glassware, serological

pipettes, fume hoods, scales, centrifuges, microscopes, plus heavier machinery. "This is one of our main analytic tools, a combination spectrophotometer and fluorometer," Packard says of a large piece of equipment. "You find this in practically every chemistry lab in Europe, so we have one too."

Hanczyc has been synthesizing and studying various types of vesicles, and today Packard wants to show me what they look like. Packard is a big man with shaggy blond hair, glasses, and a courtly manner. He has a slow and deliberate style of speech, which includes a precise, mellifluous Italian, courtesy of his wife, Grazia Peduzzi, who was born in Milan. He squints through a fluorescence microscope, adjusts the focus, and finally, there they are: the real-life correlates of the objects he had been simulating on the computer.

"Somewhat dried up," he says of the vesicles, which Hanczyc had prepared a while ago.

A vesicle is not a living thing. It's just a shell, a husk, the merest framework of the full artificial cell that's supposed to assemble itself on the premises and spring into life at some undefined point in the future. Nevertheless, what we have here on the microscope stage is something passably astonishing, slight and rudimentary though it might appear at first glance. For these filmy minute blobs are the first stirrings of an event that last took place billions of years ago: the genesis of life.

THE DREAM OF creating life has ancient roots in the human imagination. In *Frankenstein*, which Mary Shelley completed in 1817 at the age of nineteen, the scientist Victor Franken-

stein cobbled together a creature from body parts he'd spirited away in the dead of night from graveyards, dissection rooms, and slaughterhouses. The resulting beast came to life when Dr. Frankenstein, by unspecified means, infused "a spark of being into the lifeless thing that lay at my feet."

Serious scientific attempts at infusing a "spark of life" into inanimate flesh go back at least to Luigi Galvani's discovery in 1771 that by applying electrical currents to a dissected frog's legs he could cause them to twitch as if alive. A hundred years later, in 1871, Darwin spoke of life as possibly having arisen "in some warm little pond, with all sorts of ammonia and phosphoric salts, lights, heat, electricity, &c., present."

As if following Darwin's recipe, when twentieth-century scientists approached the problem of understanding how life originally arose on earth, they attempted to re-create what they thought were the original prebiotic conditions. The canonical effort, now a cliché of twentieth-century science history, was the 1952 "Urey/Miller experiment," in which the chemists Harold Urey and Stanley Miller put ammonia, hydrogen, and methane inside a closed flask, circulated steam through this "atmosphere," and added bolts of "lightning" in the form of periodic electrical sparks. All they got for their trouble were some amino acids (building blocks of proteins) that were not in the mixture to begin with. The Urey/Miller experiment was once considered a very big deal, but it isn't by some of the protocell project's scientists: "We are not searching in the black and hoping that something happens," says the protocell researcher Uwe Tangen. "We're really trying to engineer these things."

Attempting to build an artificial cell is hardly a new idea in

biology, but the specific protocell design Packard and Hanczyc are working on originated with Packard's longtime friend, the Los Alamos physicist Steen Rasmussen. Even as a boy in Denmark, Rasmussen liked to grapple with the big questions. He was by nature of a metaphysical turn of mind, and while still a kid he discussed subjects of cosmic import with his father, who was a bricklayer. Did the universe have a beginning—or an end? Where did it come from? Where was it going?

Later, in the 1980s, Rasmussen, together with Chris Langton, Norman Packard, and some others, became one of the founding fathers of the artificial life (ALife) movement. Launched at a Los Alamos workshop in 1987, artificial life was an attempt first to simulate and then actually to create a new life-form. Supposedly there was to be "soft," "wet," and "hard" artificial life, existing in the form of software, wet chemistry, and robotics, but the reality of the situation turned out to be quite different. "Most of the activities in the artificial life community have been with simulations," Rasmussen admits.

For a long time, that was true even of Rasmussen himself, who over the years had run countless computer simulations of various life-forms, modeling their possible self-assembly routes, evolutionary development pathways, and so on. But his abiding passion had always been to understand what life was and how it arose. At length he decided that the best way to understand life was to make some of it himself, ab initio.

In truth, he became obsessed with the idea. Although he lived in an adobe-style house surrounded by a number of natural life-forms, including his wife, Jenny, and three kids—not to mention horses, chickens, a parakeet, dog, and cat—he did most of his thinking at the Los Alamos lab and on his daily

commute to and from, a route that took him past some of the most inhospitable, sun-blasted terrain imaginable: desiccated red cliffs, dry desert sands, and, occasionally, the whited bones of dead animals.

So daunting was the goal of creating new life, he realized, that only the simplest and most radically stripped-down design would have the remotest chance of actually working. Any given entity, in Rasmussen's view, had to have three main attributes in order to be considered alive: it had to take in nutrients and turn them into energy, meaning it had to have a metabolism; it had to reproduce itself; and its descendants had to be able to evolve by means of natural selection. A conventional biological cell, which did all that and more, was a masterpiece of complexity: it had an outer wall through which various essential substances were selectively transported in and out. It had an inner wall around the nucleus, which did the same. And both the nucleus and the cytoplasm surrounding it were brimming with all sorts of enzymes and other biochemicals, plus microstructures and organelles: the ribosomes, the mitochondria, the Golgi bodies, and all the rest. So very complex were even the simplest biological cells that it was a wonder they worked at all.

Rasmussen didn't want to get bogged down with all that incredible complication and detail, so he set about devising "the most lousy, simple, self-replicating, autonomous unit you can imagine," a cell so small it would be "the size of dust."

He got rid of the DNA, the nucleus, the organelles, and much of the rest of standard cell wetware. His protocell would be a *minimal living entity*, thousands of times smaller than a biological cell, and would be composed of three main structures:

a container made of fatty acid molecules; a primitive metabolic system; and a new type of genetic material called PNA.

Fatty acids were also known as lipids, and their major attraction for Rasmussen was that "they make the containers for free. You put them in water and they make the containers. That's the state they want to be in. They want to join up and make these structures." The component molecules did this on account of their chemical polarity: one end of the molecule was hydrophobic (or water-avoiding), the other hydrophilic (or water-seeking), and so when placed in water the molecules naturally arranged themselves into little sponge-like vesicles with the hydrophilic ends forming the outside surfaces and with the hydrophobic ends huddled together on the inside. (Many of the protocell's activities would be governed by the twin forces of hydrophobia and hydrophilia.)

For genes, Rasmussen needed a molecule that could both contain hereditary information in the manner of DNA or RNA, and could replicate, but without having to go through all the biochemical, biomechanical, and other enzyme-driven contortions those molecules underwent in natural cells. What he needed, in short, was a coding molecule that could unzip and replicate in some quick and dirty, no-sweat, E-Z fashion. For this he chose PNA, peptide nucleic acid, a substance synthesized in 1991 by Peter Nielsen, the Danish biochemist. This was a double-stranded molecule that could split down the middle, just like DNA, uncovering its A, T, C, and G bases. Its advantage for Rasmussen, however, was the different ways in which the double-stranded and single-stranded versions of PNA behaved in the cell. A double-stranded stretch of it was hydrophobic and would sink down

into the interior of the container and away from the water that surrounded the cell. At a preset temperature, the PNA molecule would spontaneously separate lengthwise inside the cell. The bases of the two single strands were hydrophilic and would therefore rise up to the cell's outer surface. There they would encounter matching PNA fragments that were also floating in the surrounding water, placed there with malice aforethought by the experimentalists. Those fragments would now attach themselves to the single strands, thereby forming new double-stranded molecules which, hydrophobic once again, would sink back down into the cell's innards. That took care of gene replication.

The protocell's metabolic, growth, and self-reproduction processes would be a product of light-sensitive lipid molecules being force-fed into the container. Light would activate the polarity of the molecules in such a way that their hydrophilic ends would rise to the container's surface and squeeze themselves in and among the other molecules that made up the cell's exterior. When the quantity of those surface molecules reached a certain critical mass, the forces holding them together would be overcome and the cell would split in half, reproducing itself.

Natural selection would come into the picture as the protocells reproduced: those that possessed some selective advantage in the speed or efficiency of replication would displace and ultimately wipe out those deficient in those qualities.

That was the basic design plan and operating formula of Steen Rasmussen's protocell. An ingenious design by any standard, especially if it worked. But in order to put his plan into effect, the wee matter of funding had to be addressed.

"I am doing this because I want to understand what life is," Rasmussen said. "That's the driver. Now that's not enough to get money, so the secondary driver is of course, Well, how can this be useful?"

From a practical point of view, there were three key benefits to Rasmussen's protocells. One was their relative safety: because artificial cells would be structurally and chemically alien to modern biology, they would be far less risky to experiment with than genetically engineered biological cells. As strictly nonbiological entities, "they'd have a much harder time interacting with modern life," Rasmussen said. "They'd be much less of an environmental or health hazard."

Second was their controllability: since they were designed to be programmable, the scientists ought to be able to coax the protocells to perform a larger range of tasks than was possible using ordinary cells and conventional biological engineering techniques. Suitably programmed protocells could unpollute the environment. They could act as "living pharmaceuticals," adapting themselves to a given individual's changing medical needs. They could produce new fuels, chemicals, structures, materials, and technologies.

Finally, because of the commercial value of those activities, they might even—unlike most other research projects financed by the government—make a profit.

AS IT TURNED OUT, money for such a far-fetched project was relatively easy to come by in Europe—provided that you and your organization were based there. Plus, office space for part of the effort was available for free in Venice, as Norman

Packard learned while winding up his previous career in Santa Fe.

Norman was at this stage well into what might be called his Third Major Career Cycle (there were also smaller epicycles). Packard, who happened to be a cousin of David Packard, cofounder of Hewlett-Packard, had started out as a fairly conventional physicist, winning his Ph.D. at the University of California, Santa Cruz, in the late 1970s, after which he pursued a course of research into the main problem areas of the day: chaos theory, self-organizing systems, artificial life. That was his First Career Cycle. (As an epicycle to which, he and his friend Doyne Farmer designed and built miniaturized computer systems that were able to predict, fairly reliably, where a roulette ball would land after a spin of the disk. Never averse to making money with physics, Packard, together with Farmer and Mark Bedau, all of whom had been friends since their undergrad days at Reed College, secreted these devices on themselves and brought them into the casinos of Nevada—until they were busted by the gaming authorities.)

Later, Packard and Farmer founded the Prediction Company, a financial-markets consulting firm in Santa Fe. After several years of successfully modeling, anticipating, and forecasting the allegedly "unpredictable" behavior of the stock market, the business had made small fortunes for both of them. That was Packard's Second Career Cycle. At that point, "it seemed like the right time to try and break loose and pursue some other agendas," he said.

The agenda for his Third Career Cycle was established at a meeting with Rasmussen, Bedau, and their friend John

McCaskill, a theoretical chemist from Sydney, Australia, who by that time had occupied several prestigious academic posts in Germany. The meeting was held at a villa in the Italian resort town of Cannobio, on Lago Maggiore, at the foot of the Swiss Alps. It was about a hundred miles from Lake Geneva, where Mary Shelley had gotten the idea for and started writing *Frankenstein*. The villa was owned by the family of Packard's wife, Grazia.

By the time of this gathering in the summer of 2002, these four researchers—Packard, Rasmussen, McCaskill, and Bedau—were old friends, and had closely shared scientific interests, orientations, and ambitions. Ever since a similar meeting two years before at Ghost Ranch in New Mexico (the former home of artist Georgia O'Keeffe, which was later made into a conference center), the four of them had become increasingly fixated on Steen Rasmussen's protocell design plan. Now, after a week's worth of discussions in Cannobio, the band of brothers decided that the time had come to implement Steen's design. They laid out an organizational plan, a timetable for action, and an informal division of labor, and then they mutually pledged themselves to actually building a protocell.

When they arrived in Cannobio, they were four investigators in search of a project. By the time they left they were the Four Protocell Musketeers. Then they disbanded, each to carry out his allotted part of their overall vision.

First, John McCaskill would apply to the European Union for a grant to establish an international consortium to be known as PACE, an acronym for Programmable Artificial Cell Evolution. Its primary and ultimate objective would be