Can Synthetic Biology Shed Light on the Origins of Life?

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Abstract
It is a most commonly accepted hypothesis that life originated from inanimate matter, somehow being a synthetic product of organic aggregates, and as such a result of some sort of prebiotic synthetic biology. In the past decades, the newly formed scientific discipline of synthetic biology has set ambitious goals by pursuing the complete design and production of genetic circuits, entire genomes, or even whole organisms. I argue that synthetic biology might also shed some novel and interesting perspectives on the question of the origins of life, and that, in addition, it might challenge our most commonly accepted definitions of life, thereby changing the ways we might think about life and its origins.

Keywords
definition of life, origins of life, prebiotic chemistry, synthetic biology

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The origins of life remain one of the most puzzling unanswered questions of science. Several decades after Schrödinger's *What is Life?* (1944), Haldane's *The Origin of Life* (1929), and Oparin's (1924) book with the same title, new insights have been gained into this ever more challenging question, but nothing close to a definite answer has been won. Today, synthetic biology presents itself as a novel scientific discipline, somehow at the borderline between biology and engineering, whose objective is to engineer biological systems in radically novel ways: "synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature" (Serrano 2007: 1). By engineering living systems and by deconstructing and reconstructing novel forms of life synthetic biology comes close to the frontier of living and non-living matter. For some, "synthetic biology . . . attempts to recreate in unnatural chemical systems the emergent properties of living systems" (Benner and Sismour 2005: 533). Could synthetic biology thereby shed some new light on the old question of the origins of life? In this contribution, I will argue that this is indeed the case, even if these new insights might still be far from providing a full answer. To start with, I will propose a delineation of the discipline of synthetic biology, broadly construed, by looking at the types of problems it aims to solve. I will then consider the question of the origins of life so as to make explicit the types of problems that need solving from this perspective. This will make it possible to understand how and to which extent synthetic biology might contribute to solving this most fascinating puzzle. I will also argue that synthetic biology, because it is committed to creating novel forms of life, is likely to make another indirect contribution to this question, namely that of challenging our definition of life.

Delineating Synthetic Biology

Contemporary synthetic biology certainly has little to do with the synthetic biology of the late 19th century as advocated by Moriz Traube, Stéphane Leduc, and the like (Keller 2002, and this issue). Methods and tools have changed, no doubt. Yet the name remains, and probably also one common objective: the synthesis of complete living organisms from non-living matter. By trying to chemically reproduce the biological phenomenon of mitosis, Leduc, for instance, pursued the objective of synthesizing cellular artificial organisms. Such a synthesis remains an objective of contemporary synthetic biology, even if it has somehow been sidetracked by shorter-term ones. The synthetic biology of today somehow encompasses, redefines, and broadens biotechnology (Koide et al. 2009): some of its ultimate goals are to design and build complete biomolecular and genetic systems that react to specific signals, process them, and produce desired outputs. The latter include informational signals, chemical compounds, molecular structures, energy, and nutrients that could improve food production, enhance human health, and preserve the environment. For some, the term synthetic biology "is used to describe the wholesale engineering of genetic circuits, entire genomes, and even organisms" (Lucentini 2006: 30). I will use this definition as a starting block to delineate synthetic biology as it is pursued today along three major dimensions that capture the objects manipulated by this discipline as well as the types of problems it pursues. I therefore propose to identify three types of synthetic biology.

Synthetic Biology as Engineering of Genetic Circuits (Type I Synthetic Biology)

By interacting with one another through the mediation of other molecular entities such as RNAs or proteins, genes can form complex webs of interactions that may be referred to as *genetic networks* or *genetic circuits*. Like electronic circuits, genetic circuits may have positive or negative feedback loops as well as linear and nonlinear interactions. Unlike electronic circuits, genetic circuits are not wired, as molecular interactions take place in aqueous solutions. In any case, one of the peculiarities of all such circuits is their dynamics (the way they behave with time), which is often quite difficult to decipher from the networks' mere static description. There is obviously a long tradition in biology to study such networks as they appear in nature, and their behavior over time (e.g., Jacob and Monod 1961). Yet, the *de novo* design and production of genetic networks is a much more recent endeavor and a major focus of synthetic biology. Its objective is no longer the discovery and explanation of naturally occurring genetic circuits, but the complete engineering of networks that behave according to plan. This is what I propose to label "Type I synthetic biology."

Examples include the design and production of synthetic oscillatory networks (e.g., Elowitz and Leibler 2000; Sprinzak and Elowitz 2005; Stricker et al. 2008; Tigges et al. 2009), of bistable switches (e.g., Gardner et al. 2000; Kim et al. 2006), or even the exploration of stabilizing features such as autoregulatory loops (e.g., Becskei and Serrano 2000). Sometimes an existing genetic circuit also is "rewired," i.e., made to respond to another molecular signal (see, e.g., Dueber et al. 2004). Generally, the desired function is realized by inserting specific genes into an existing cell, say, a bacterium; these genes are then expected to make the cell behave in a specific way that corresponds to the dynamics of the newly created genetic circuit. Take the example of an oscillatory network that makes bacteria periodically synthesize fluorescent proteins in addition to all their regular activity: the artificial engineering network that is added to the genetic circuitry of the bacteria makes the bacteria perform the function of a visible clock (Elowitz and Leibler 2000).

The aim behind this kind of research is to design (from scratch) a genetic circuit that will perform a specific task within

a given living organism without interfering with its regular functioning—typically its self-sustaining metabolic activity and capacity to reproduce. Hence the goal of engineering "biobricks" (e.g., Endy 2005) that can perform sets of given functions and may be used as modules to give rise to even more complex behaviors, somehow similar to the way electronic components such as transistors can be integrated into a circuit. There is, then, a strong analogy between this type of synthetic biology and electronics, which is reinforced by the desire to build catalogs of standardized parts such as the Registry of Standardized Biological Parts (http://partsregistry.org). In addition, there is a need for well-understood organisms within which such biobricks can be inserted and can be made to work in the desired way. This is the role of "chassis organisms" that are flexible and versatile enough to express a wide variety of foreign genes, while somehow retaining their core functional integrity (see, e.g., Metzgar et al. 2004).

Synthetic Biology as Engineering of Entire Genomes (Type II Synthetic Biology)

Synthetic biology also covers research work that does not concern specific genetic circuits but complete genomes. The objective in this case is no longer the design and implementation of a given function within an organism by means of sets of biobricks, but the *de novo* synthesis of whole genomes that can then be made to work, typically by inserting them into a cell whose nucleus has been emptied of its original genetic material. I will refer to this type of work as "Type II synthetic biology." In such cases, a complete synthetic genome is produced *in vitro* from readily available industrial nucleic acids that are assembled into a sequence very similar to the sequence of wild-type organisms.

Examples range from the *de novo* chemical synthesis of the 7,500 base-pair RNA genome of the smallpox virus (Cello et al. 2002) to that of the 580,000 base-pair DNA genome of *Mycoplasma genitalium* (Gibson et al. 2008). These projects can also be related to complete genome exchanges between species of organisms (e.g., Lartigue et al. 2007) and genome simplification and redesign (e.g., Chan et al. 2005). In the case of *Mycoplasma genitalium*, for instance, the synthesis involved five major steps: (1) the chemical synthesis of a hundred oligonucleotides of some 6,000 base pairs each; (2) patching these oligonucleotides four by four into 24,000 base-pair DNA strands by *in vitro* polymerase chain reaction (PCR); (3) amplification and cloning into *E. coli*; (4) further patching into 72,000 and 144,000 base-pair strands by the same techniques; and (5) the final patching of these longer strands by transformation-associated recombination (TAR) cloning in the yeast *S. cerevisiae*. The main challenges of such projects appear to consist in the assembly, manipulation, and cloning of such large DNA sequences. Yet, what is lurking behind is the capability to chemically synthesize living organisms from scratch. Even if one might be reluctant to qualify viruses as living organisms, and even if the synthesis of the genome of *Mycoplasma genitalium* is not the synthesis of a complete organism, the possibility of completely synthesizing living systems appears closer than ever before. Incidentally, such research projects also open up the possibility to investigate the viability of organisms whose genomes have been significantly altered and reduced, thereby examining the conditions required for carrying out the essential vital functions, as would be the case with a minimally reduced *Mycoplasma genitalium* genome of some 350 genes (Hutchison et al. 1999).

Synthetic Biology as Engineering of Organisms (Type III Synthetic Biology)

In addition to devising genetic circuits and synthesizing complete genomes, synthetic biology also includes research that aims at engineering complete novel living systems from scratch. I will refer to this type of synthetic biology as "Type III." In this case, the objective is not really to copy nature and build organisms that would be duplicates or modifications of naturally occurring ones, but rather to investigate the self-organizational properties of matter at the transition from inanimate matter to life. The goal that this type of synthetic biology pursues is the *in vitro* assemblage of chemical systems that are capable of metabolic activity and self-maintenance, reproduction, and variation. In a way, such chemical systems would be partly alive, depending on the functions they would be able to carry out.

Examples include research on self-assembly amphiphile molecules, lipids, or fatty acids that can spontaneously self-assemble into micelles or bilayer vesicles, the properties of which might include growth, budding, division, fusion, catalysis of the formation of other vesicles (e.g., Bachman et al. 1992; Monnard and Deamer 2002), or even catalysis of the synthesis of RNA-like polymers, possibly showing how an early prebiotic coupling might have appeared between lipids and a form of primitive genetic polymer (Rajamani et al. 2008). Other examples of research in this area include the tentative assembly of protocells by having catalytic RNAs encapsulated into vesicles (e.g., Szostak et al. 2001), or even starting from a cell-free protein synthesis (Noireaux et al. 2005). Others also propose the assembly of lipid aggregates that might be capable of integrating at the same time proto-genes and a proto-metabolism (e.g., Rasmussen et al. 2003).

In such instances, Type III synthetic biology appears to manipulate simpler, smaller molecular objects than Types I and II do. It also manipulates these objects with the aim of bridging non-living and living matter in radically novel ways: rather than inserting (networks of) biobricks into existing life forms, or chemically synthesizing complete genomes and organisms copied from current ones, Type III synthetic biology aims at investigating the chemical synthesis of the simplest possible

protocells from readily available organic compounds. To do so it relies on two major activities: the design and engineering of such protocells or chemical systems on the one hand, and on the other, the tentative implementation of these designs and processes into "wet chemistry."

Questions on the Origins of Life

Research on the origins of life aims at explaining the transition from inanimate matter to life, which is supposed to have taken place in the early ages of our planet, some four billion years ago. One would think that the rock record would provide unique evidence of what happened at that time. Yet its interpretation does not give unambiguous answers. Indeed, Archean molecular fossils remain puzzling and their putative biological origins hard to establish. Nevertheless, despite unsettled controversies about the origins of such fossils and differing interpretations, it appears reasonable to believe that living systems already existed on Earth some 3.5 billion years ago (Brasier et al. 2006; Schopf 2006). This would leave a few hundred million years for life to appear on Earth. Now the question is: *how* did life originate?

Contemporary research on the origins of life encompasses a fairly broad spectrum of approaches, from prebiotic chemistry (chemistry that is supposed to be compatible with the environmental conditions of primitive Earth) to molecular biology, theoretical biology, and artificial cell engineering, as well as contributions from geology, micropaleontology, and planetology that define historical and environmental constraints. The main challenge is that of bridging non-living and living matter in conditions compatible with those that are estimated to have been those of the early Earth (e.g., Kasting 1993, 2005). In other words, how can we explain that the simple molecules that were available in the cosmos and on primitive Earth ended up generating quite complex functional sets of chemical compounds capable of life? I argue that this quest for explanation rests on three major components (see Figure 1): (1) the identification of *prebiotic molecular entities*, (2) the specification of *prebiotic evolutionary processes* that explain how the different molecular entities have been produced, and (3) the specification of *functioning mechanisms* that explain how primitive living organisms carry out the different functions that would make them alive.

Prebiotic Molecular Entities

The successive sets of molecular entities that are thought to have existed from those available on the primitive Earth up to the appearance of fully functional protocells are precisely those that I propose to refer to as "prebiotic molecular entities." These objects are nothing but molecules and sets of molecules that happen to play a crucial role in the explanation of the appearance of life on Earth. In particular, four major classes

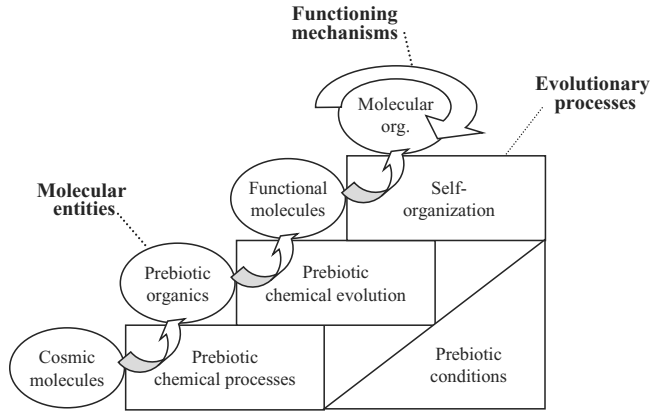

Figure 1.
Three major explanatory components of origins of life theories.

of such life-relevant molecules can be defined, by order of chemical size, complexity, and appearance (see Figure 1).

Obviously, one has to start with the molecules that are supposed to have been readily available on the very early Earth or in space at that time. These *cosmic molecules* typically encompass simple compounds such as methane, ammonia, or water. From these, one should be able to explain the appearance of *prebiotic bricks*: these are the first organic molecules synthesized in prebiotic conditions. They include amino acids, simple peptides, sugars, bases, nucleic acids, nucleotides, lipids, and so on. A second step should then explain the appearance of *functional molecules*, typically polymers and assemblies of prebiotic bricks into larger compounds that possess puzzling properties such as that of cross-catalysis. Such molecules would include oligopeptides, short RNA strands, as well as other genetic and catalytic polymers that might have preceded RNA. A third step would then lead to the very first functional molecular organizations (some would say *protocells*). These supramolecular assemblies are sets of interacting functional molecules. They might, for instance, consist in autocatalytic networks that might be coupled with lipid vesicles; as such, they might be taken as the first signs of life.

In order to bridge non-living and living matter, any explanation of the origins of life needs to appeal to each of these four classes of molecular entities. One of the requirements is also that such molecular entities should be compatible with the chemical and environmental conditions of primitive Earth in terms of atmospheric composition, pH, temperature, water solvency, and so forth.

Prebiotic Evolutionary Processes

The second component required for a satisfactory explanation of the origins of life consists in being able to put forward evolutionary processes that can account for the transitions from one type of molecular entities to the next, up to the very first protocells, under the constraint that these processes be totally

abiotic and compatible with the environmental conditions of the early Earth. At least three major classes of evolutionary processes need to be put forward in order to gradually step from cosmic molecules to protocells.

The first one consists of sets of *prebiotic chemical processes* that would account for the synthesis of prebiotic bricks from readily available cosmic molecules. Prebiotic chemical processes of this kind typically consist of complex sets of chemical reactions, organized in networks of linked reactants and products, also determined by specific chemical conditions. Examples include Miller's synthesis of amino acids by applying an electric discharge to a mixture of CH_4, NH_3, H_2O, and H_2 (Miller 1953; Bada and Lazcano 2003), the synthesis of nucleic bases, such as adenine, from a mixture of cyanidric acid HCN and ammonia NH_3 (Oró and Kimball 1961; Ferris and Orgel 1966; Orgel 2004), or from a eutectic solution of HCN (Schwartz et al. 1982), among many others.

A second one consists of a *principle of prebiotic evolution* that might justify the appearance of more complex organic molecules with specific functional properties. In other words, such a principle would explain how prebiotic functional molecules appeared from sets of prebiotic bricks. As the *in vitro* synthesis of artificial catalytic RNAs, also called "ribozymes" (Bartel and Szostak 1993; Johnston et al. 2001), indicates, such a principle of prebiotic evolution might consist of rounds of random chemical synthesis of assemblies of prebiotic bricks, selection of some of them for specific catalytic activities or for increased stability, followed by their multiplication either by cross-catalysis (Lifson 1997) or by differential molecular survival (de Duve 1987), possibly coupled with chemical variation processes.

A third explanatory component consists of *prebiotic self-organization processes.* Such processes aim at explaining the appearance of the first signs of organization, be they structures (e.g., vesicles) or functional systems (like self-maintained autocatalytic networks), under prebiotic conditions. Examples of structural self-organization processes include the physical explanation of the spontaneous formation of vesicles in solutions containing amphiphile molecules under certain conditions of concentration, pH, and temperature (Hargreaves and Deamer 1978; Bachman et al. 1992; Monnard and Deamer 2002). Explanations have also been put forward to account for properties of such membranes including the appearance of selective molecular exchanges (Sacerdote and Szostak 2005), growth, budding, fusion or fission properties (Hanczyc and Szostak 2004), or surface catalysis properties (Rajamani et al. 2008). Examples of functional self-organization include cross-catalytic nucleic acids (Yjivikua et al. 1990), cross-catalytic RNAs (Sievers and von Kiedrowski 1994; Kim and Joyce 2004), and cross-catalytic sets of oligopeptides (Lee et al. 1996; Yao et al. 1998; Ashkenasy et al. 2004). Even if they are not yet successfully realized *in vitro*, it is believed that such processes of self-organization, possibly combined with one another, coupled and integrated, might lead to an explanation of the appearance of fully functional protocells, i.e., chemical systems somehow capable of self-maintenance and reproduction with variation.

Functioning Mechanisms

A third component necessary to explain the origins of life is an account of how a protocell carries out the different properties that make it "alive," for only an account of the functioning mechanisms of such protocells will allow to fully understand what it takes to be living. In other words, in addition to explaining how one bridges non-living and living matter on longer time scales in terms of prebiotic evolutionary processes, it also appears necessary to understand how a living protocell functions on its own, shorter, time scale.

Such functioning mechanisms might include complete models of protocells once these protocells have been successfully produced *in vitro*. They might resemble protocell models like those of the "chemoton" (Gánti [1971] 2003) or "autopoietic system" (Maturana and Varela 1973) that describe how protocells might be able to function. The chemoton, for instance, is a minimal cell model that is composed of three stoichiometrically coupled autocatalytic subsystems: a metabolic system that produces the molecular compounds required for the self-maintenance of the cell; a template replication system that duplicates the information required for metabolism and regulation; and a continuously renewed and growing membrane that encloses the two other subsystems. Such functioning mechanisms should make it possible to understand how a protocell carries out all the different properties that somehow make it alive.

Synthetic Biology and the Questions on the Origins of Life

Can synthetic biology shed light on the origins of life? To answer this question, let us assess to which extent each one of the three types of synthetic biology might provide keys to each of the three challenges related to the question of the origins of life.

Prebiotic Molecular Entities Seen from Synthetic Biology

In most of synthetic biology, there is little or no interest in the origins of the molecules and molecular assemblies that are used as experimental starting blocks. What matters above all is the functioning of the biological systems that are assembled or synthesized. As a matter of fact, these molecular entities can have multiple origins: they might be extracted from living organisms or artificially synthesized in man-made chemical processes. In synthetic biology, their origin is of little concern

compared to the functional success or failure of the biological systems that they are components of—as far as molecular entities are concerned, "anything goes" provided it works. As a consequence, the prebiotic relevance of these same molecular entities is also of little or no interest. In other words, if some molecules can do the job they were intended to, the fact that they might be synthesized by prebiotic chemical processes or not is not relevant. This is definitely the case for some of the most significant projects carried out in synthetic biology. For instance, in their design and production of a genetic oscillatory network, Elowitz and Leibler (2000) cloned known nucleotide sequences via PCR so as to build specific plasmids that were subsequently introduced into a given strain of *E. coli*. Obviously, the steps of this experimental process as well as the molecular entities upon which it rests have no prebiotic relevance: the process of PCR is totally artificial; the DNA strands of interest have specific sequences that come from extant living organisms; the final assemblies consist in living bacteria that have been modified by introduction of plasmids. None of these elements might be qualified as prebiotically relevant, and in this respect Type I synthetic biology sheds no light on the origins of life.

In a similar fashion, when Venter, Hutchison, and Smith synthesized the entire genome of *Mycoplasma genitalium*, they made use of modern technologies to assemble "cassettes" of several thousand base pairs each, of *in vitro* PCR amplification and cloning into *E. coli* for patching the cassettes together, and of TAR cloning in *S. cerevisiae* for final assembly of the genome. Obviously, in this Type II example none of these experimental steps might have spontaneously happened on primitive Earth. On the other hand, one could argue that the sets of genes that would be identified as necessary and sufficient for a minimal living cell base on the same biochemistry as that of current living organisms (e.g., Hutchison et al. 1999), could bring valuable insight with regards to the question of the origins of life. Of course, being already an extremely sophisticated mechanism of some 250–300 genes, such a minimal genome would not be the backbone of some of the earliest forms of life. Nevertheless, it might provide a most relevant evolutionary milestone in between the origins of life *per se* and the simplest and most primitive life forms we currently know of.

Type III is probably the area of synthetic biology that is most concerned with the prebiotic relevance of the molecular entities it manipulates. For instance, when Szostak, Bartel, and Luisi propose to synthesize protocells by encapsulating catalytic RNAs into vesicles, one of their concerns is the relevance of their assemblies as hypothetical steps in the transition from non-living to living matter (Szostak et al. 2001). Indeed, even if they carefully warn that "solutions found in the laboratory need not be chemically similar or even directly relevant to the actual molecular assemblies that led to the origin of life on Earth" (p. 387), they place their work within the framework of the *RNA world* hypothesis, according to which self-replicating RNA strands might have constituted the very first forms of life (Gilbert 1986), and conclude: "experimental possibilities could provide fascinating insights into what is now a complete black box of early evolution" (p. 390). Their protocells include two types of molecular entities: on one hand an RNA replicase, and on the other, lipid molecules that can self-assemble spontaneously into vesicles. Both types might bear prebiotic relevance, the first within the hypothesis of the RNA world, the second within the *lipid world* scenario that emphasizes the appearance of amphiphile molecules on primitive Earth and their self-assembly into vesicles that can grow, bud, divide, and fuse (Segré et al. 2001). In such cases, then, synthetic biology might be able to shed some light on the origins of life, not so much by explaining the origins of the molecular entities that constitute the building blocks of its experimental research, but by giving an account of how such molecular entities and their self-assembly might have resulted in primitive life forms, while ensuring that these building blocks were compatible at least with some scenarios of prebiotic chemistry.

Evolutionary Processes Seen from Synthetic Biology

The identification of the prebiotic evolutionary processes that might have contributed to the gradual transition from non-living to living matter is probably also not the prime focus of synthetic biology as a whole, even though, as we will see, some work in this area (especially from Type III synthetic biology) might partly contribute to this aim.

Indeed, it is probably fair to say that Type I and Type II synthetic biology do not shed much light on such prebiotic evolutionary processes, whose discovery is not one of their goals. For instance, the research teams that work on engineering genetic circuits (Type I) do not provide much insight into any hypothetical prebiotic evolutionary process. When Elowitz and Leibler (2000), Stricker et al. (2008), or Tigges et al. (2009) engineer synthetic genetic oscillatory networks, their research does not point to any such evolutionary process. Rather, they focus on designing and successfully implementing specific functions, in these cases genetic oscillators, independently of any constraint or research question that might have to do with the origins of life. The only "evolutionary processes" that are of interest in this kind of work are the dynamic evolutionary trajectories of the genetic systems that were engineered. For instance, the focus of the project described by Stricker et al. (2008) consists in the design of a genetic oscillatory network and the study of its dynamics over time. Of particular interest are the large-amplitude fluorescence oscillations that persist throughout observation runs as well as the oscillatory period of the network that can be tuned by altering inducer levels, temperature, and media source. This dynamic behavior is also at stake when comparisons are made with computational models.

Such comparisons may point to key design principles for constructing robust oscillators, e.g., a time delay in the negative feedback loop, or the effect of positive feedback as a means of increasing the robustness of the oscillations, or implementing greater tunability (Stricker et al. 2008). Such dynamic behaviors, however, are characterized over rather short time periods and do not entail drastic changes to the systems they describe. As such, they do not correspond to the longer-term evolutionary processes that aim at explaining the progressive transition from non-living to living matter and which, by doing so, would entail strong changes to the systems they might apply to. (Let us recall that such prebiotic evolutionary processes aim at explaining how organic molecules might have emerged from simpler widely available chemical compounds, how functional polymers might have appeared from sets of random monomers, or more generally how supramolecular assemblies with life-like properties might have self-organized from mixtures of prebiotic molecules.)

In a similar fashion, Type II synthetic biology is also not much concerned with prebiotic evolutionary processes. When synthesizing genomes, Cello et al. (2002) as well as the research team of Venter, Hutchison, and Smith (Gibson et al. 2008) focus on the production of an end result—a target genome. What matters therefore is whether the sequencing of the synthetic genome matches the target genome or not. To this end, any experimental process can be used to assemble nucleotides into oligonucleotides and subsequently into DNA strands and complete genomes: PCR amplification, plasmid introduction and cloning into living bacteria, TAR cloning, and so forth. Obviously, such processes bear no relevance to the prebiotic processes that might have occurred on primitive Earth and led to the first forms of life.

The perspective is slightly different, I will argue, in the case of Type III synthetic biology. Indeed, when scientists investigate how lipids or fatty acids might spontaneously self-assemble into micelles or bilayer vesicles that are then capable of growth, division, or fusion (e.g. Bachman et al. 1992; Monnard and Deamer 2002), they often have in mind the possible prebiotic relevance of such processes. For instance, for Bachman, Luisi, and Lang, "because of the simple mechanisms underlying their spontaneous formation, aqueous micelles are plausible candidates for the first self-replicating bounded structures" (1992: 59). Similarly, Monnard and Deamer qualify the process of spontaneous self-assembly of bilayer vesicles as "steps towards the first cellular life" (2002: 1996). In such cases, the experimental processes that lead to the formation of micelles or vesicles are thought to be good candidates for prebiotic processes that would explain the appearance on primitive Earth of similar structures. Also, when Deamer and his team show how RNA-like polymers can be synthesized non-enzymatically from mononucleotides in lipid environments and how such RNA-like polymers might end up encapsulated within lipid vesicles, they claim that "this process provides a laboratory model of an early stage of evolution toward an RNA World" (Rajamani et al. 2008: 57). And when Szostak et al. (2001: 390) propose to synthesize protocells by encapsulating catalytic RNAs into vesicles, they claim that "by supplying a population of protocells with random RNA sequences, one might observe the process of evolving complexity in real time." Examples of Type III synthetic biology can be found, then, that stress the relevance of their work in terms of processes that might shed light on some of the prebiotic evolutionary mechanisms.

Functioning Mechanisms Seen from Synthetic Biology

There seems to be two competing approaches in synthetic biology with regard to understanding the functioning mechanisms of the biological systems under investigation. On one hand, some appear to favor a black-box approach. In this case, what matters is not so much the understanding of the mechanisms that produce a given result as the very result itself. As Madrigal (2008: 1) puts it, "it's not what you learned, but what you made." An example might be that of Venter and his team who harnessed a certain number of techniques to achieve their goal of artificially synthesizing the complete genome of *Mycoplasma genitalium* (Gibson et al. 2008). One of these techniques is to use the yeast *S. cerevisiae* for the final assembly of four huge DNA strands into the final genome of *Mycoplasma genitalium*. While this process works, little is known about the mechanisms that make it work and typically also why this type of assembly is not possible within the bacterium *E. coli* that was used in earlier steps of the same experiment to stitch together smaller segments of DNA. Similar examples can be found all across synthetic biology. For instance, one could argue that when a genetic oscillator is identified and works within a given organism, one does not fully understand why it does so, and why in contrast the same genetic oscillator would not work in another closely related organism (Serrano 2007). And in a similar fashion, when Deamer and his team show that RNA-like polymers can be synthesized non-enzymatically from mononucleotides in lipid environments and that such polymers can become encapsulated within lipid vesicles (Rajamani et al. 2008), they show that such things do indeed work the way they do, yet they do not claim to fully master the underlying explanations of these phenomena. Therefore, wherever such a black-box attitude pertains, synthetic biology is unlikely to yield a detailed explanation of the functioning mechanisms of hypothetical primitive living systems, and this is likely to be the case in any of the types of synthetic biology I have identified.

A competing view can be found, however—the view that takes after Feynman and according to which "what I cannot create, I do not understand" (see, e.g., Hawking 2001; see also

O'Malley, this issue). In this case, understanding how things work the way they do is also a key component of the research agenda. Take, for instance, the projects that aim at assembling protocells from catalytic RNAs and lipid vesicles (e.g., Szostak et al. 2001). Of course, the main objective is to produce such protocells and to make sure *that* they work—grow, divide, evolve, etc. Nevertheless, a secondary objective is also to understand *how* these protocells work. Three elements may contribute to this understanding: (1) a detailed knowledge of the parts constituting the system that is to be built, in this case specific strands of RNA, and specific lipid molecules; (2) knowledge of the assembly process and conditions that lead to this very system, e.g., in terms of molecular concentration, pH, or temperature, but also in terms of chemical and thermodynamic processes that govern the self-assembly of the lipid molecules into micelles or vesicles of different shapes, the engulfment of RNA strands, and so forth; and (3) knowledge of how the different molecular entities dynamically interact and evolve over time once the protocells have been assembled, leading, e.g., to their growth, budding, fission, or fusion. If such projects are pursued and successfully realized, synthetic biology will indeed be able to provide very valuable insights on the functioning mechanisms of specific types of protocells, and thus on the possible functioning mechanisms of hypothetical primitive life forms—provided, of course, that the molecular components of these protocells and the experimental conditions that lead to their formation be compatible with the chemistry of prebiotic Earth.

Indirect Perspectives on Life

Overall, it appears that synthetic biology might be able to shed *some* light on the origins of life, and that contributions are somehow more likely to come not so much from Type I synthetic biology (engineering of genetic circuits) but from Type II (engineering of complete genomes), and most of all from Type III (engineering of organisms). In this respect, insights may be provided on some of the later stages of the emergence of the first living organisms, yet not so much on the earlier stages, i.e., the prebiotic synthesis of the necessary molecular compounds and the range of evolutionary processes that could explain the complete transition from non-living to living matter. In addition—and because synthetic biology is all about novel life forms—I argue that it can bring another major indirect contribution to the quest of the origins of life, viz., expanding life and redefining its boundaries.

Novel Living Forms

If synthetic biology is about the "engineering of biology" and the "synthesis of novel functions" (Serrano 2007), it is also, *de facto*, about the creation of novel life forms, viz., life forms that do not exist in nature and that are not the result of Darwinian evolution. When novel genetic circuits (Type I synthetic biology) are implemented in existing organisms (be they *E. coli, S. cerevisiae*, or *Acinetobacter*, to name but a few), when complete genomes are synthesized (Type II) and genomes of certain organisms replaced by those of others, or when novel organisms are the overt objective of research projects (Type III), novel living organisms are *de facto* created. Of course, the extent to which these organisms are "novel" depends on the degree of their modification when compared to the most closely related natural organisms. In this respect, an *E. coli* that has been supplemented with a fluorescent "repressilator" (Elowitz and Leibler 2000) will appear less new than the much desired protocell (Szostak et al. 2001). The point is that synthetic biology keeps creating novel life forms that might result from addition or modification of genetic circuits, from genome simplification or more drastically from complete novel synthesis. As a consequence, synthetic biology is expanding the realm of known life.

This expansion can be pictured as going in three directions. The first is "sideways": when current living organisms are modified by insertion of engineered genetic circuits (e.g. Elowitz and Leibler 2000), or by a rewiring of existing pathways (e.g. Dueber et al. 2004), the result are organisms that are somehow related to the natural organisms they once were and their degree of complexity, hence the number of functions they might carry out is also similar. The second is "downward" in the sense of less complexity and simpler ancestry. This is the case when, for instance, genome reductions are carried out to investigate the functional limits of minimal genome sets (e.g. Hutchison et al. 1999). Such genome reductions expand the realm of life by offering insights into simpler organisms than those existing today, and incidentally, into organisms that might bear some relatedness to ancestral, DNA-based, cellular life forms. The third direction that synthetic biology might expand known life is what might be called "fast-downward"—somehow moving the barrier of life beyond minimal DNA-based cells into even simpler protocells (e.g., Szostak et al. 2001), and in this case, possibly offering glimpses into the very first forms of life that appeared on Earth.

Redefining Life

By expanding the realm of life in these three directions, synthetic biology is also bound to contribute to a broader philosophical question of (re)defining life (many definitions of life have been proposed; see, e.g., Sagan 1970; Luisi 1998; Palyi et al. 2002: 15–56; Popa 2004: 197–205). Indeed, by engineering novel life forms, synthetic biology offers insights into novel ways of delineating what is alive from what is not. In particular, the engineering of minimal organisms, be they simplified versions of current ones or radically novel systems, might challenge some of the traditional dichotomous definitions of life according to which any given system is either alive or not.

As a matter of fact, there is disagreement about whether certain biochemical systems should qualify as living or not. Viruses are a classic example: some authors (e.g., recently, Luisi 1998; Ruiz-Mirazo et al. 2004) argue that viruses should not count as living systems because they lack metabolic activity, whereas others (e.g., Raoult and Forterre 2008) argue they should, in particular when they form "viral factories." Moreover, some scientists argue that self-replicating strands of RNA as hypothesized in the RNA world scenario (Gilbert 1986) may count as being alive as they are capable of replication and variation (e.g., Luisi 1998), whereas others argue the contrary, invoking the lack of metabolic activity and membrane enclosure (e.g., Shapiro 1986; Segré et al. 2001). And the debate goes on. In a similar fashion, one could question whether minimal protocells as proposed by Szostak et al. (2001) or Libchaber and his team (Noireaux et al. 2005) might more properly qualify as alive than, say, those pursued by Rasmussen and colleagues (2003).

Beyond a dispute about where the "true" demarcation between living and non-living matter really is, the creation of novel borderline biochemical systems might also indicate that such a clear-cut demarcation simply does not exist. Rather than being an "all-or-none" issue, the transition from non-living to living matter might very well be more properly qualified as a "more-or-less" question. In other words, the predicate "to be alive" would be better captured in fuzzy logic as a continuum along a zero-to-one scale rather than in first-order classical logic (Bruylants et al. in press). Furthermore, synthetic biology could end up producing novel organisms that might be not only "more-or-less alive" but also "more-or-less alive in different ways," i.e., alive along several dimensions or modes that might correspond to the functioning diversity of such living systems. For instance, a system could be more or less successful at reproducing itself, or more or less successful at metabolizing components or energy tokens from given sets of available compounds, or even more or less successful at individuating itself by means of more or less robust and sophisticated membranes (Malaterre in press).

As a result, by expanding the domain of known living organisms, synthetic biology might end up producing different "types" of living systems, and by the same token might point to different scales and modes for biochemical systems to be alive. Life might then not be a matter of "yes or no" but of "modes of being alive" and of "degrees" along these modes. Even if synthetic biology is not there yet, this discipline may recast the way we think about life and help redefine this fundamental concept.

Conclusion

In this contribution, I proposed to review the extent to which synthetic biology might shed light on the origins of life. Synthetic biology has been mapped out as consisting of three main types of activities: engineering of genetic circuits (Type I), engineering of complete genomes (Type II), and engineering of organisms (Type III). On the other hand, I have argued that the question of the origins of life can be split along three different dimensions: (1) the prebiotic relevance of the molecular entities that are manipulated, (2) the identification of prebiotic evolutionary processes, and (3) the specification of functioning mechanisms. Overall, it appears that synthetic biology is not much concerned with the origins of prebiotic organic compounds; at best some projects of Type III can be considered as taking into consideration the prebiotic relevance of the molecular components of their biochemical systems. It is also mainly Type III synthetic biology that might offer some glimpses of possible prebiotic evolutionary processes in so far as they might be derived from the kind of *in vitro* self-assembly and molecular evolution that pertain to the projects Type III synthetic biology is interested in. Finally, some hypotheses about the functioning mechanisms of primitive life forms might be derived from work on minimal genomes within Type II synthetic biology, as well as on protocells such as those targeted by some Type III projects. Overall, therefore, and even if this is not its prime focus, synthetic biology might indeed be able to shed some new light on the origins of life. Its major contribution, however, might be that of challenging our current dichotomous delineation of life from non-life: indeed, by synthesizing novel forms of life, synthetic biology is bound to produce an increasing number of borderline biochemical systems that might show, if anything, that life is not a matter of yes-or-no but a matter of "modes of being alive" and of "degrees" along these modes. Even if the direct contribution of synthetic biology to the question of the origins of life might appear somehow limited, radically changing the way we view life is no little achievement.

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