Orig Life Evol Biosph (2011) 41:3–16
DOI 10.1007/s11084-010-9214-1

The Role of Natural Selection in the Origin of Life

Iris Fry

Received: 9 March 2010 / Accepted: 6 April 2010 /
Published online: 21 April 2010

Abstract It is commonly accepted among origin-of-life scientists that the emergence of life was an evolutionary process involving at one stage or other the working of natural selection. Researchers disagree, however, on the nature of the chemical infrastructure that could have formed prebiotically, enabling the evolutionary process. The division of the origin-of-life research community into 'geneticists' and 'metabolists' usually revolves around the issue whether the first to arise prebiotically was a genetic polymer or a primitive metabolic system. In this paper I offer an alternative classification based on the attitude to the onset of natural selection. From this perspective I add to the conventional division between gene-first and metabolism-first groups a position I call "preparatory metabolism". By this line of thought, an RNA or an RNA-like polymer could not have emerged prebiotically. Nevertheless, the onset of natural selection had to wait until such a polymer had arised. This paper examines the RNA-first, RNA-later, metabolism-first and preparatory-metabolism scenarios, assessing the weaknesses and strengths of each. I conclude that despite the recent theoretical advances in all these lines of research, and despite experimental breakthroughs, especially in overcoming several RNA-first hurdles, none of the examined paradigms has yet attained decisive experimental support. Demonstrating the evolvability of a potentially prebiotic infrastructure, whether genetic or metabolic, is a most serious challenge. So is the experimental demonstration of the emergence of such an infrastructure under prebiotic conditions. The current agenda before origin-of-life researchers of all stripes and colors is the search for the experimental means to tackle all these difficulties.

Keywords Digital and analog information · Gene-first · Metabolism-first · Natural selection · Origin of life · Protometabolism

I. Fry (✉)
Department of Humanities and Arts, Technion—Israel Institute of Technology, Technion City, Haifa 32000, Israel
e-mail: iris.fry@gmail.com

Introduction

It is commonly accepted today by origin-of-life researches that the emergence of life was an evolutionary process involving, at one stage or another, the working of natural selection. The early systems, constituting the Last Universal Common Ancestor at the root of the evolutionary tree, were already highly complex. They could not have been the product of regular physical and chemical processes alone and had to be the consequence of evolution. Hence, an infrastructure of specific chemical structures, enabling an evolutionary process, had to arise on the primordial earth. Such infrastructure, as made clear by Cairns-Smith (2008), had to combine two features. First, unlike living systems that are products of and participants in evolution, these prebiotic chemical structures were not products of evolution. Not being yet intricately organized, they could have emerged as a result of ordinary physical and chemical processes. Second, these structures were evolvable, i.e., capable of evolution by natural selection.

While convinced of the need for evolution in the emergence of life, researchers disagree on the nature of the required chemical infrastructure that could have formed on the primordial Earth.

Based on mechanisms active in extant cells, many scientists adopt a "gene-first" position. This view assumes that the starting point for evolution consisted of a genetic polymer of some sort, probably an RNA-like or maybe even an RNA molecule (Gilbert 1986; Joyce and Orgel 2006). The "gene-first" position is not weakened by within-camp debates whether RNA was the first prebiotic genetic polymer or whether it was predated by a chemically simpler polymer (Anastasi et al. 2007; Powner et al. 2009). Rejecting the genetic view, alternative theories claim that life emerged through the evolution of autocatalytic, metabolic cycles constituted by small organic molecules (Lancet and Shenhav 2009; Pohorille 2009; Shapiro 2007; Smith and Morowitz 2004; Wächtershäuser 1992). Opponents of the gene-first conception argue that the synthesis of RNA or its analogs and their replication were extremely improbable under prebiotic conditions and that the emergence of life could have been based on a non-genetic foundation.

The division between "geneticists" and "metabolists" as discussed in this paper differs from its usual analysis in the literature. Most often, theories that deny the early emergence of a genetic polymer are grouped together under the title of "metabolism-first", notwithstanding their position on the stage at which natural selection entered the emergence process. For example, de Duve's ideas, which I categorize under "preparatory metabolism", are mistakenly grouped under "metabolism-first" because of his denial of the prebiotic emergence of RNA (see, Anet 2004, 654; Orgel 2000, 12503; Orgel 2006; Shapiro 2007, 50; Shenhav et al. 2003, 20). This classification ignores de Duve's position that a genetic polymer is necessary for the onset of natural selection (De Duve 2005a). Also, defying the conventional classification, Cairns-Smith rejects as improbable the early emergence of organic building blocks and organic genetic polymers but is nevertheless a "gene-first" proponent (Cairns-Smith 2008, 3836). He suggests an evolutionary process based on the self replication of prebiotically abundant crystal 'genes' that eventually gave rise to RNA polymers (Cairns-Smith 1982; Cairns-Smith 2008, 3837).

The fundamental debate on the true nature of the required prebiotic infrastructure for natural selection is somewhat obscured by other controversies dividing the field, e.g., the "soup" versus a hydrothermal-vents environment, and autotrophic versus heterotrophic origin (Deamer 2008; Orgel 2006; Russell and Martin 2004). As important as these controversies are, a closer look reveals that the division between "geneticists" and "metabolists" does not run parallel to these fault lines (De Duve 2003, 560–561; De Duve 2005b, 155–157; Russell and Martin 2004).

The objective of this paper is to examine how genetic and metabolic theories account for the evolutionary nature of the emergence process and to try to assess the strength of their positions. To limit the scope of this wide subject, only chosen few examples of mostly recent research are examined. Though it is clear that the questions raised here will be settled eventually by experimental evidence, a small step in this direction might be gained by a clarification of the terms of the debate.

The Gene-First Position—Current Assessment

The gene-first position is shared today by many, some would claim most, researchers in the field. Its proponents are divided into two camps. One insists on the RNA-first option, according to which RNA could have been a "prebiotic product" (Anastasi et al. 2007, 721), that is, could have emerged by physical and chemical means to constitute an infrastructure for the emergence of life. The second group claims that only simpler genetic polymers could have emerged by prebiotic chemistry, later to evolve and "invent" RNA (ibid.). The "RNA-later" option is the result of several decades of failed experiments to show that the components of an RNA-world could have formed prebiotically. (Anastasi et al. 2007, 721; Joyce and Orgel 2006, 24; Orgel 2004, 115).

The chemical steps to be accomplished prebiotically for the RNA-first option to materialize were enormous in number and complexity. In principle, the right monomers had to be synthesized and polymerized, and a ribozyme capable of self-replication, an RNA replicase, had to be present within the available "library" of oligonucleotides. This would have constituted a necessary, though not a sufficient condition for the initiation of Darwinian evolution process culminating in the Last Universal Common Ancestor. In the 2006, 3rd edition of their 1993 seminal paper on the origin of RNA, Joyce and Orgel still referred to "the molecular biologist's dream" promised by the RNA-first possibility and to the grim reality of "the prebiotic chemist's nightmare" frustrating such promise (Joyce and Orgel 2006, 23–24).

And Yet, RNA-First

A few recent experimental developments in the prebiotic synthesis of RNA are lending support to RNA-first hypothesis, of which one notable example is discussed: Until recently, all attempts to synthesize ribose under plausible prebiotic conditions and to then add it to a nucleobase have proved either inefficient (with purines) or impossible (in the case of pyrimidines). Eschewing the "dogma" (Anastasi et al. 2007, 724) that ribonucleotides should be assembled from their seemingly obvious molecular components, a new method bypassed free ribose and nucleobases, using instead a common precursor for both, in the presence of phosphate, and leading to the synthesis of pyrimidine ribonucleotides (Powner et al. 2009; Szostak 2009). Though many remaining problems are pointed out, such as the need to find a method for the synthesis of purine ribonucleotides, the authors feel that by "thinking outside the box" a plausible way for the prebiotic synthesis of RNA might be discovered (Anastasi et al. 2007, 724–5). Similar attempts to adopt an unconventional approach to old problems that were made by other RNA-first researchers also seem to advance the RNA-first goal (e.g., Breslow and Cheng 2009; Kanavarioti et al. 2001; Klussmann et al. 2006; Pino et al. 2008; Ricardo et al. 2004).

Assuming a successful synthesis of RNA monomers and their polymerization to produce a pool of random-sequence RNA molecules, the daunting question of the presence in this

pool of a self-replicating ribozyme still remains unresolved. Despite the impressive catalytic capabilities of natural ribozymes isolated so far from living cells, no catalytic polymerase has been detected. At the same time, in vitro evolution and isolation of ribozymes capable of various functions has become now a routine laboratory procedure (Joyce 2004). So far, the most promising isolated ribozymes relevant to the polymerization of RNA act as ligases that catalyze the template-directed binding of two shorter oligoribonucleoties to produce a copy of the template (Bartel and Szostak 1993). Such ligases are then used in directed-evolution experiments to isolate catalytic RNAs with novel and more potent properties. Recently, ribozymes functioning as more efficient RNA polymerases have been isolated (Johnston et al. 2001; Zaher and Unrau 2007).

Joyce and colleagues have managed to evolve a self-replicating ligase ribozyme. By structuring the ligase as a symmetrical dimer, the ligated product was identical to the template (Paul and Joyce 2002). Using in vitro evolution of the cross-replicating ligases and selecting for their improved catalytic versions, these authors attained much faster replication times and the possibility of many rounds of replication. Sequence variation in the cross-replicating ribozymes and recombination between variants led to the rise of dominant recombinant replicators. Due to different binding affinities, preferred pathways for certain mutations are expected. Thus, such a population of RNA enzymes demonstrates a simplified model of a genetic system (Lincoln and Joyce 2009, 1229, 1231).

For supporters of the RNA-first option these recent developments strengthen the prospect of an experimental realization of their scenario, whereas opponents point to the huge hurdles that still loom large (Orgel 2004, 115). Some researchers claim that these experiments might be irrelevant for the prebiotic emergence of life, being dependent on the design and active manipulation by the experimentalists (De Duve 2005b, 74; Orgel 2008, 11; Shapiro 2007, 49).

On the Need for Enclosure

One of the noticeable recent advances in gene-first research is the emphasis on the role of membrane-enclosed compartments in the emergence of a genetic infrastructure for Darwinian evolution (Deamer 2008; Szostak et al. 2001).

Whereas early gene-first theories assumed a “naked gene” to arise by chance on the ancient Earth (Haldane 1967[1929], 247; Oparin 1965b, 96; Troland 1914), the first metabolists, notably Oparin and later Fox, saw the emergence of a primordial metabolism within an enclosed space, a colloidal “coacervate” or a microsphere made of protein-like polymers, as the decisive first step on the way to life (Fox 1984; Oparin 1953[1936], 148–160). Current metabolists follow the same direction (Morowitz 1992, 1999; New and Pohorille 2000; Segré et al. 2001). Wächtershäuser suggests a protective membrane on his mineral-based “surface metabolist” (Wächtershäuser 1992, 104–106). On the other hand, just a few years ago, Orgel still supported the idea of “naked genes” absorbed on the surface of mineral particles, doubting whether early membranes could be permeable enough to allow the passage from the environment of small organic molecules and still retain internally replicating macromolecules (Orgel 2004, 117). However, experiments have now demonstrated this possibility (Mansy et al. 2008), and the need for early compartments, in one way or another, has become a majority view in the origin-of-life field.

An RNA replicase ribozyme engulfed by a membrane that can grow and divide was suggested in a model for the Darwinian evolution of primitive cells (Chen et al. 2004; Szostak et al. 2001). The tight interaction between genome and membrane characteristic of a living cell was proposed to form through the evolution of another RNA ribozyme that catalyzes the synthesis of membrane components (Szostak et al. 2001).

More recently, higher concentration of RNA within fatty-acid vesicles was found to entail a higher internal osmotic pressure and to lead to the growth of membranes by obtaining additional membrane material from other isotonic vesicles. Difference in the efficiency of RNA replication within vesicles could have thus led to competition among vesicles and to the evolution of a superior replicase. If this mechanism proves valid, Darwinian evolution of such cells could have emerged on the basis of a one-gene cell (see, Chen et al. 2004, 1476).

"RNA-Later"

Notwithstanding the recent developments examined here, many gene-first proponents doubt whether RNA arose prebiotically and prefer to search for a simpler-than-RNA genetic polymer. (Orgel 2003, 213; Orgel 2004, 118).

Many candidate genetic polymers were studied, among them RNA analogs whose backbone is made of sugars other than ribose linked by phosphate groups (Eschenmoser 1999; Eschenmoser 2007a, 12823–12827). Another class of polymers consists of peptide nucleic acids (PNAs), whose backbone is made of amide rather than ribose-phosphate bonds (Nielsen 1993; Nielsen 2009). Another suggested option are alanyl nucleic acids (ANAs) in which the oligonucleotide analogs include D- and L-alanine in an alternating arrangement (Diederichsen 1996). This unique structure enables the formation of a stable pairing between, e.g., Oligo Gs and oligo Cs.

All these polymers were examined for their ability to form stable double helices and to cross-pair with RNA strands. Though some of the analogs demonstrated both properties, it is not clear whether they might have been easier to synthesize prebiotically, compared to RNA (Anastasi et al. 2007, 733–736; Orgel 2004, 114–115). Moreover, the evolution of these various chemical "worlds" to the RNA-world, including the emergence of the required catalysts, presents unlikely scenarios (Anastasi et al. 2007, 737). Thus, it is not clear yet whether these polymers can be considered as serious candidates for an earlier genetic material (but see, Nielsen 2009, 337–338).

Various genetic polymers not made of nucleotide monomers were also considered. Amino acids that could have been easily synthesized prebiotically were proposed as such monomers. Orgel noted the possibility of charge-dependent pairing between aspartic acid and arginine, or size-dependent between aspargine and glutamine (Orgel 2004, 116). However, a principal difficulty is the lack of nonspecific stacking interactions characteristic of nucleic-acid bases that are crucial for stability of the pairing chains (Orgel 2004).

Experiments demonstrating the self replication of peptides have been conducted since the 1990s. Several research groups demonstrated autocatalytic ligation by peptides of two shorter peptides (Issac and Chmielewski 2002; Lee et al. 1996; Lee et al. 1997). Experiments also confirmed the formation of a hypercycle in which two competitive self-replicating peptides were shown to symbiotically catalyze each other's production. Such a system resembles the previously reported hypercycle made of cooperating RNA sequences (Eigen 1992, 107–114). It raises the possibility that similarly to RNA, such peptide system could have competed with other peptide hypercycles in an evolutionary process and that like RNAs, peptides might have functioned originally as both genes and enzymes (Lee et al. 1997).

Yet and despite these advances, Orgel pessimistically summed up recent RNA-later studies saying that though the idea that RNA was "invented" by a simpler genetic system is a popular one, "no convincing precursor system has been described" (Orgel 2004, 116).

Metabolism First

What kind of infrastructure for evolution under prebiotic conditions is offered by the metabolism-first alternative? The answer, in outline, was provided already in the 1920s and 1930s by Alexander Oparin's pioneering theory. Not being aware yet, at that stage, of the molecular basis of inheritance and evolution, Oparin nevertheless described a prebiotic scenario in which chemically evolved and catalytically competent organic molecules interacted to form primitive metabolic chains within colloidal droplets. These droplets could absorb organic substrates from their environment, and depending on their internal metabolism were able to grow and divide upon reaching a certain size (Oparin 1953 [1936], 163–195).

Following inaccurate division, the "offspring" droplets, though reproducing and grossly "inheriting" their parent's organization, showed some variations. Differing in their metabolic capabilities and hence in their rate of growth and reproduction, they engaged in competition for resources. This competition resulted in the evolution of more efficient and complex enzymes within the droplets and in the production of more complex metabolites. In post Watson-Crick years, Oparin updated his scenario and experiments, allotting a role for polynucleotides (1965a, 340; Lazcano 2010, 9–10). However, he distinguished his theory from the genetic school of thought (1965a, 332). Whereas the latter argued for "the evolution of the molecule" that culminated in the production of RNA-like molecules, Oparin emphasized the "self-reproduction of the entire system" (1965b, 96) and continued to rely on a metabolic-like mode of evolution (1965a, 340).

With the updated knowledge of molecular structures and mechanisms, current metabolism-first position is formulated in more specific terms and the emphasis is on the autocatalytic nature of metabolic cycles. Still, Oparin's original model of reproduction, inheritance, variation, competition and evolution of complexity is the basis of most metabolic theories up to now. This is the case despite other dividing issues among metabolic theories, e.g., autotrophy versus heterotrophy and "soup" versus hydrothermal-vents environments (Fox 1984; Oparin 1953[1936]; Wächtershäuser 1992).

Some versions of the Oparin model were suggested by Fox (1984), by Kauffman (1993, 330–333), by Morowitz and colleagues, who applied it to the prebiotic evolution of lipid vesicles (1992, 152–154) and also to the self-replication of the reverse citric acid cycle, the hypothetical first prebiotic metabolic network, in a hydrothermal-vents environment (Morowitz 1999; Smith et al. 2009, 452). Through the reduction of carbon provided by CO_2, the completion of the cycle resulted in the doubling of its molecules. Such autocatalytic doubling is viewed in this model as a form of primitive reproduction. Differences in autocatalytic efficiencies within a population of these metabolic networks provided the basis for competition and selection (Smith et al. 2009, 445–446).

Another notable metabolic theory is Wächtershäuser's, in which the role of a closed compartment is replaced by a protective water-repellent membrane formed on the surface of pyrite crystals. Wächtershäuser's "surface metabolist", comprised of a core pyrite crystal on which organic molecules formed and interacted, also demonstrated growth-related division (Wächtershäuser 1992, 104–107).

The fundamental mechanism on which current metabolists base their case for the evolvability of autocatalytic cycles, is a positive feedback loop grafted onto such a cycle (see, Morowitz 1992,153–154; New and Pohorille 2000; Pohorille 2009, 572; Shenhav et

al. 2003, 22–32; Smith et al. 2009, 445–446; Wächtershäuser 1992, 89, 111–112; 2007, 594–5).

Wächtershäuser, for example, proclaims the highly determined emergence of auto-catalytic cycles, out of which rare "catalytic branch products" resulted in variations of the original cycle. Any "branch product" feeding back into the cycle and into its own production guaranteed "a memory effect", i.e., the basis for heredity of a variant cycle (Wächtershäuser 1994, 2007, 594, 595). Autocatalytic cycles could thus evolve toward higher complexity and toward "an increasing number of reaction possibilities" (Wächtershäuser 1994, 4286; 2007, 599–600).

Lancet and his group applied the basic Oparin tenets to their lipid-world model in which assemblies of catalytic organic molecules (e.g. amphiphiles) within protocells were able to grow, divide, and sustain the parents' "compositional information" over generations in the daughter protocells (Lancet and Shenhav 2009, 239; Segré et al. 2001, 137–139). Division also gave rise occasionally to statistical variations among progeny assemblies and to "imperfections in the catalytic networks" regarded as "compositional mutations" (Lancet and Shenhav 2009, 243).

Common to all these different versions is the attempt to offer an evolutionary scenario not based on some type of template-directed replication. Instead of an infrastructure based on "digital information" (Wächtershäuser 1994, 4285, footnote), these various theories thus consider an "analog" (Wächtershäuser 1994fs) or "compositional" (Segré et al. 2001, 137) concept of information and inheritance altogether

"Digital" and "Analog" Information

To recapitulate, according to the prevailing thesis the emergence of life, entailing the development of complex organization, depended on a process of evolution by natural selection. For such a gradual process to succeed, any advantageous feature achieved at one stage had to be handed over, or memorized from one generation to the next. This is why the unique chemical properties of nucleic acids: their ability to form specific hydrogen bonds between their bases that enable their faithful copying, are so attractive and so difficult to give up even as the earliest genetic systems (Chu and Orgel 1999, 448; Eigen 1992, 64–67)

A central debate in the context of evolution and development is the question of digital versus analog information. Maynard Smith (1998), Dawkins (1995) and others have claimed that only digitally encoded information can assure long-term fidelity, a prerequisite of biological inheritance. Explaining why in order to function as genetic material, genes have to be digital, Dawkins said that in distinction to discrete symbols, analog information, because of its continuous nature, tends to be degraded with repeated copying as is the case with recopied tapes and photocopies of photocopies. "Genes, on the other hand, can self-copy for ten million generations and scarcely degrade at all" (Dawkins 1995, 19).

Sterelny and Griffiths, on the other hand, pointed to the fertilized egg as a convincing example of functioning analog system. The various chemical gradients in the egg, they argued, are "analog instructions" that guide the process of cell differentiation in the early embryo. According to these authors, "this information…is reconstructed with high fidelity generation by generation", with the help of error correction mechanisms that might work also in other analog systems (Sterelny and Griffiths 1999, 368–370, see also, Pohorille 2009, 573, 578; Lancet and Shenhav 2009, 239, 241).

Notwithstanding the controversy over the nature of present-day cells, are Dawkins's and similar pro-digital arguments applicable to the origin of life? Could it be that an analog,

metabolic system had a better chance than a digital, genetic polymer, to emerge on the early Earth, and once formed didn't have to withstand the very long test of eons of geological time, as did biological evolution? An inaccurate reproduction of such a metabolic system which still provided an infrastructure for natural selection could have functioned for a limited period of time, giving rise to more complex chemical structures. These structures could have later evolved into more accurate genetic systems. Also, though an error correction mechanism (Sterelny and Griffiths 1999, 370) is obviously not a strong argument in the case of the origin of life, one could imagine some type of "memory", or "fidelity" becoming possible by physical and chemical constraints exerted by prebiotic conditions. These constraints might have channeled the system repeatedly to a specific analog state.

Morowitz and his colleagues contend that the very first organized systems to emerge and engage in prebiotic evolution were constrained and "selected" by geochemical conditions on the early Earth (Smith and Morowitz 2004, 13168; Smith et al. 2009, 452). Strict constraints also characterize Wächtershäuser's theory (Wächtershäuser 2007, 594). Wächtershäuser claimed that in the highly constrained prebiotic situation there must have been "a paucity of possibilities for the first autocatalytic cycle and for its early autocatalytic expansion. In the most extreme case there may be only one chemical possibility for such a first cycle and for the early sequence of expansions of this cycle. In this sense, it may be considered chemically determined" (1994, 4286; see also 2007, 587).

What is the Plausibility of a Prebiotic Metabolic Cycle?

One of the strongest arguments in favor of metabolic scenarios is their alleged much higher probability compared to the extreme improbability of the prebiotic emergence of a self-replicating genetic polymer (Shapiro 2007). This argument was recently criticized by a few researchers (Anet 2004; Pross 2004). Orgel repeatedly returned to the question of the plausibility of self-organizing biochemical cycles with the motivation to explore their role in the emergence of genetic self replication (Orgel 2000). Though a committed 'geneticist', Orgel's position within the genetic camp was unique. Along with Crick and Woese he independently suggested the RNA-world idea already in the late 1960s (Orgel 1968). While being a leading contributor to the development of the RNA-world theory, he was also one of its most severe critics (Joyce and Orgel 2006). Thus, his views of the metabolic option were not those of a mere partisan.

Orgel chose to analyze the plausibility of the prebiotic self-organization of the reductive tricarboxylic acid (rTCA) cycle as proposed by Morowitz (Smith and Morowitz 2004) and Wächtershäuser (1988). He raised the question whether such a nonenzymatic cycle could have formed with the help of prebiotic catalysis. Examining the various reactions involved in the cycle (Orgel 2000, 2008) he reached the conclusion that although it is very likely that minerals were instrumental in prebiotic chemistry, it is highly unlikely that one mineral (Wächtershäuser's pyrite) functioned as a specific catalyst for several unrelated reactions (Orgel 2000, 12506). Morowitz and colleagues did not commit themselves to a specific catalyst, but assumed that the various reactions depended on "the type of catalysts that [were] available" (Smith et al. 2009, 448), probably "in the vicinity of catalytic transition metal surfaces" (452). Orgel contended that the chances that specific minerals catalyzing each of the reactions will occur at a single locality "in the absence of catalysts for disruptive side reactions seems remote in the extreme" (2008, 8).

In return, Morowitz and colleagues acknowledged that empirical analysis of possible side reactions had to be conducted, but insisted that taking into account various chemical

and thermodynamic factors the reverse citric acid cycle was plausible. They pointed out the empirical demonstration of several stages relevant to the cycle even without specific mineral catalysis (Smith and Morowitz 2004, 13172–3; see also Smith et al. 2009, 444).

Orgel also analyzed the much-discussed metabolic model suggested by Kauffman (Kauffman 1986). According to this model, out of a collection of short peptides, capable of catalyzing the ligation of other peptides, a subset can organize to form a cycle in which each member is catalyzed specifically by one or more other members of the subset. The cycle as a whole is thus self-replicating (Kauffman 1986). As in the case of Morowitz's and Wächtershäuser's metabolic theories, Orgel's major criticism of Kauffman's model was of ignoring the lack of catalytic specificity of short peptides. Without such specificity, Orgel argued, a peptide-cycle theory is not plausible (Orgel 2008, 11).

"Preparatory Metabolism"

Similarly to other metabolists, Christian de Duve rejected as highly implausible the prebiotic "invention" of RNA (1991, 112–113). Finding the option of a simpler pre-RNA genetic polymer to be without evidence in extant organisms and unhelpful in terms of prebiotic chemistry (2005b,73, 75), de Duve suggests the prebiotic emergence of catalytic "multimers", peptide-like substances synthesized with the help of thioester bond energy, that constituted a growing protometabolism (2005b, 15–24). However, de Duve does not regard this protometabolism as providing an infrastructure for natural selection, which he claims could begin only when ribonucleotides were synthesized and polymerized (2005a). The frequent inclusion of de Duve's theory together with those of Kauffman, Morowitz, Wächtershäuser, Lancet and others thus misses a crucial distinction (see, Anet 2004, 654; Orgel 2000, 12503; Shapiro 2007, 50).

De Duve's multimers, unlike genetic polymers, were not supposed to replicate. Unlike other metabolic scenarios, his is not based on the reproduction of catalytic cycles. What is thus de Duve's alternative means of "memory" that guaranteed the prolonged activity and development of protometabolism? First, De Duve claims that protometabolism must have relied on "a set of *robust* reactions capable of being maintained during the time… needed for enzyme-catalyzed metabolism to arise" (2003, 562). He also believes that without the help of selection "chemical determinism had to suffice" (2005b, 24). Due to stringent constraints dictated by the chemistry of different amino acids and other monomers and the properties of the resulting multimers, the multimer mixture was nonrandom and reproducible as long as environmental conditions did not change (1991, 140–145; 2003, 568; 2005b, 87).

Crucially, de Duve makes the claim that protometabolism and metabolism were *congruent*. Early protometabolic chemistry must have acted as a "screen" in selecting the first metabolic enzymes and ribozymes. Only on the basis of existing substrates and outlets for waste products could enzymes be useful and thus selected. At the same time, protometabolic reactions were carried over into metabolism only if enzymes catalyzing them arose and were selected (2005b, 19–21, 152). Though believing that the synthesis of RNA was "a true watershed in the development of life" (2005b, 87) de Duve does doubt, however, whether in the RNA world all the chemical reactions necessary for the early systems were carried out by ribozymes (83). Protometabolism probably continued to function unchanged until protein enzymes evolved (84–87).

It is worthwhile to compare de Duve's and Orgel's ideas on the possible steps leading to the RNA world. Interestingly, examination of their positions reveals common ground

between their seemingly different perspectives. Based on theoretical and experimental considerations, Orgel came to think that the RNA world might have been preceded by evolvable genetic polymers constructed from simple monomers, easily synthesized prebiotically, e.g., amino acids. He argued that in order to be able to synthesize nucleotides, "pre-RNA 'organisms'…must have developed fairly on sophisticated 'enzymes' prior to the transition to the RNA world" (2003, 213). Similarly to de Duve, Orgel then suggested that some of these 'enzymes' continued to function in the RNA world side by side with ribozymes and only ultimately were taken over by protein enzymes (2003, 213–214, 217).

Hence, both Orgel and de Duve conceived of the early evolution of life in terms of a replicating polymer. Yet, it was Orgel, the 'geneticist', who did not limit evolvability to RNA and was ready to consider a 'peptide organism' as an early biological system, i.e., a system capable of replication and evolution. For de Duve, the protometabolic stage was not evolvable, persisting in its stable existence due to chemical determinism until giving rise to RNA.

Another representative of what I call "preparatory metabolism" is the geochemist Michael Russell, who proposed that life emerged inside iron and nickel sulfide compartments naturally arising near submarine hydrothermal vents (Russell and Hall 1997). Unlike Wächtershäuser's metabolic-first scenario, based on a metabolic infrastructure for reproduction and evolution, Russell and William Martin don't see the need for a cyclic CO_2 fixation, e.g., the reverse citric acid cycle (Martin and Russell 2003, 66). They favor instead the linear primitive acetyl-CoA pathway catalyzed by metal sulfides as the first exergonic biochemical pathway (Russell and Martin 2004). Russell and Martin hypothesize that this reaction produced organic compounds that participated in a primordial biochemistry catalyzed by the metal-sulfide-made inner surfaces of the compartments (Russell and Martin 2004). This geochemical confinement prevented the diffusion of organic products into the ocean. Metabolism, supported by the accumulated, concentrated organic molecules, eventually developed into an RNA world (Martin and Russell 2003, 65–66). Russell is currently putting his scenario to the test, attempting the synthesis of methane and acetate by the mixing of an acidic, cold "ocean" with alkaline, hot spring water (Whitfield 2009).

Analogous to de Duve's suggested robust protometabolism, Russell's and Martin's scenario is chemically determined under stable environmental conditions. They argue that "a hydrothermal reactor would contain, *stably over geological time*, a good set of continuously produced ingredients" (Russell and Martin 2004, 5, emphasis mine).

Conclusions

How successful are the RNA-first, RNA-later, metabolism-first and, preparatory-metabolism theories in elucidating the emergence of life as an evolutionary process?

So far, none of these paradigms can claim a decisive experimental support. Yet, at least in principle, experimental approaches directed by gene-first theories provide a strong potential for achieving their goal. No functioning system of genetic replication has been achieved yet, unless an external protein enzyme has been added to the system (Hanczyc et al. 2009, 121). However, based on recent experiments, the RNA-first scenario seems to make some advance in this direction (Hanczyc et al. 2009, 121; Lincoln and Joyce 2009; Paul and Joyce 2002, 12739; Szostak et al. 2001). RNA-later theorists believe that some of the many genetic polymers under study might have a similar potential (Cleaves 2009, 600–605; Nielsen 2009, 338).

The experimental task facing metabolism-first theories is much more difficult, as even metabolists themselves acknowledge (Lancet and Shenhav 2009, 234; Pohorille 2009, 569). On the one hand, the prebiotic synthesis of a pool of small organic molecules, including some endowed with catalytic capabilities, is much more plausible than the preparatory stages of genetic models. On the other hand, metabolists face the enormous challenge of demonstrating, first, that a subset of such a pool could have closed to form an autoctalytic cycle and, second, that a group of such cycles could have evolved. Not all metabolists agree whether at all these tasks, especially the second, can be experimentally demonstrated (Lancet and Shenhav 2009, 245–247; but see Shapiro 2007, 52). The synthesis of a number of key biomolecules and the establishment of several metabolic reactions that could have formed part of core metabolic chains were achieved by Wächtershäuser and Huber (Huber et al. 2003; Huber and Wächtershäuser 2006, 630; but see Ross 2008) and by Cody et al. (2001, 2004). This work does not relate yet to the question of evolvability.

So far, most metabolic theories are not based on experimental evidence but rather on computer models and simulations of the formation of metabolic cycles and their reproduction (see, Kauffman 1986; New and Pohorille 2000; Segré et al. 2001). Computer models and simulations are often regarded by experimentalists only as supporting tools and not as substitute to experimental results (Anet 2004, 656; Lancet and Shenhav 2009, 247; Pross 2004, 312; Shapiro 2007, 51). Most metabolists, however, consider such simulations as valid demonstration of the possibility of metabolic self-organization (Smith et al. 2009, 445), of "non genomic evolution" (New and Pohorille 2000) and of "compositional genomes" (Lancet and Shenhav 2009, 242–3; Pohorille 2009, 570). No one, however, including in-silico proponents (Mavelli and Ruiz-Mirazo 2007, 458; Pohorille 2009, 578), doubts that only with the support of experimental results there is chance for progress and for winning over the origin-of-life community to the case of metabolism-first (Shapiro 2007, 52).

Due to the enormous uncertainty surrounding origin-of-life boundary conditions, hypotheses based in many cases more on chemical plausibility than on empirical evidence are important guiding tools in all the research programs examined here (see Anastasi et al. 2007, 737 ; Cleaves 2009, 586–587; James and Ellington 1995, 520, 524–5; Rausch et al. 2004). A notable example is the aforementioned choice made by Orgel of peptides as pre-RNA genetic polymers. Acknowledging that this hypothesis was unproved yet (Orgel 2004, 116), Orgel considered the possibility that extant biochemistry may give us some clues about the chemistry that preceded the RNA world (2004, 213). Consequently, he contended that he was "unable to suggest any broadly distributed, metabolically important biomolecules, other than aminoacids, that seem to be plausible candidates for a primitive genetic system" (2004, 217).

The claim for the evolvability of metabolic cycles rests on the hypotheses that the thermodynamics and chemical possibilities of prebiotic environment led to the emergence of autocatalytic cycles. Among such cycles, some branched out to form products that could intervene catalytically in the working of the cycle and also in their own production (Eschenmoser 2007b, 311–312; Wächtershäuser 1994, 4285; 2007, 594, 595). It is notable that when Orgel considered for the first time the evolvability of metabolic cycles as a serious option, he also suggested exploring a side reaction, out of the reverse citric acid cycle, generating a catalyst for one of the reactions in the core cycle (2008, 10). Eschenmoser also raised the hypothesis of the contingent emergence of catalytic loops feeding into existing autocatalytic cycles that could potentially turn a metabolic cycle into a genetic one (2007b, 311–312). Eschenmoser pointed out, though, that only when a given autocatalytic cycle can be shown to operate experimentally, it will be possible to assess or

discover the formation of catalysts "feeding back into the cycle" (Eschenmoser 2007a, 12838; see, also, Orgel 2008, 10–11). Furthermore, after positively considering possible features of an evolutionary process based on a metabolic infrastructure, Eschenmoser nevertheless reaffirmed the outstanding advantages of catalytic informational polymers compared to cycles of small organo-catalysts (2007b, 313). Thus, similarly to Orgel (Orgel 2008, 5, 12), Eschenmoser regarded the most probable function of metabolic cycles not as evolvable systems but as contributors to the emergence of elementary informational oligomers (2007b, 313).

In a sense, this brings us back to the project of preparatory metabolism. Indeed, under the conditions of a robust enough protometabolism (De Duve 2003) and a stable enough geological environment (Russell and Martin 2004) guaranteeing the prolonged activity of protometabolism, the production of metabolites and small organo-catalysts could have helped to overcome the obstacles on the road to RNA. In this case, experiments guided by the tenets of preparatory metabolism (see, de Duve 2003; Whitfield 2009) could be crucial also from the gene-first perspectives.

The origin-of-life field has come a long way since its beginnings in the 1950s. The extent and sophistication of knowledge in all the scientific areas relevant to the emergence of life on Earth have grown tremendously. At the same time, the naïve optimism of the early decades was replaced by the realization of the "immense distance in knowledge and competence" still separating us from understanding the problem (Eschenmoser 2007a, 12821). This realization is shared by both geneticists and metabolists (Joyce and Orgel 2006; Orgel 2004, 119; Pohorille 2009, 578), who, as indicated here, are nevertheless attempting in their various ways to narrow this distance. This insistence stems from the common conviction that the emergence of life was a physico-chemical process which, as such, will sooner or later yield to a scientific elucidation (Fry 2000, 215–216).

References

Anastasi C, Buchet FF, Crowe MA, Parkes AL, Powner MW, Smith JM, Sutherland JD (2007) RNA: prebiotic product, or biotic invention? Chem & Biodivers 4:721–739

Anet F (2004) The place of metabolism in the origin of life. Curr Opin Chem Biol 8:654–659

Bartel DP, Szostak JW (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411–1418

Breslow R, Cheng Z-L (2009) On the origin of terrestrial homochirality for nucleosides and amino acids. Proc Natl Acad Sci USA 106:9144–9146

Cairns-Smith AG (1982) Genetic takeover and the mineral origin of life. Cambridge University, Cambridge

Cairns-Smith AG (2008) Chemistry and the missing era of evolution. Chem Eur J 14:3830–3839

Chen IA, Roberts RW, Szostak JW (2004) The emergence of competition between model protocells. Science 305:1474–1476

Chu BCF, Orgel LE (1999) Peptide-formation on cysteine-containing peptide scaffolds. Orig Life Evol Biosph 29:441–449

Cleaves HJ II (2009) Prebiotic chemistry, the primordial replicator, and modern protocells. In: Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) Protocells: bridging nonliving and living matter. MIT, Cambridge, pp 584–613

Cody GD, Boctor NZ, Hazen RM, Brandes JA, Morowitz HJ, Yoder HS Jr (2001) Geochemical roots of autotrophic carbon fixation: hydrothermal experiments in the system citric acid-H2O-(±FeS)-(±NiS). Geochim Cosmochim Acta 65:3557–3576

Cody GD, Boctor NZ, Brandes JA, Filley TR, Hazen RM, Yoder HS Jr (2004) Assaying the catalytic potential of transition metal sulfides for abiotic carbon fixation. Geochim Cosmochim Acta 68 (10):2185–2196

Dawkins R (1995) River out of Eden. Basic, New York

De Duve C (1991) Blueprint for a cell. Patterson, Burlington

De Duve C (2003) A research proposal on the origin of life. Orig Life Evol Biosph 33:559–574
De Duve C (2005a) The onset of selection. Nature 433:581–582
De Duve C (2005b) Singularities: landmarks on the pathways of life. Cambridge University Press, Cambridge
Deamer DW (2008) How leaky were primitive cells? Nature 454:37–38
Diederichsen U (1996) Pairing properties of alanyl peptide nucleic acids containing an amino acid backbone with alternating configuration. Angew Chem Int Ed Engl 35:445–448
Eigen M (1992) Steps towards life. Oxford University Press, Oxford
Eschenmoser A (1999) Chemical etiology of nucleic acid structure. Science 284:2118–2124
Eschenmoser A (2007a) The search for the chemistry of life's origin. Tetrahedron 63:12821–12844
Eschenmoser A (2007b) Question 1: commentary. Orig Life Evol Biosph 37:309–314
Fox SW (1984) Proteinoid experiments and evolutionary theory. In: Ho MW, Saunders PT (eds) Beyond Neo-Darwinism. Academic, New York, pp 15–60
Fry I (2000) The emergence of life on Earth. Rutgers University Press, New Brunswick
Gilbert W (1986) The RNA world. Nature 319:618
Haldane JBS (1967[1929]) The origin of life. In: Bernal JD (ed) The origin of life. Weidenfeld & Nicolson, London, pp 242–249, Appendix II
Hanczyc MM, Chen IA, Sazani P, Szostak JW (2009) Steps toward a synthetic protocell. In: Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) Protocells: bridging nonliving and living matter. MIT, Cambridge, pp 108–123
Huber C, Wächtershäuser G (2006) α-Hydroxy and α-amino acids under possible hadean, volcanic origin-of-life conditions. Science 314:630–632
Huber C, Eisenreich W, Hecht S, Wächtershäuser G (2003) A possible primordial peptide cycle. Science 301:938–940
Issac R, Chmielewski J (2002) Approaching exponential growth with a self replicating peptide. J Am Chem Soc 124:6808–6809
James KD, Ellington AD (1995) A search for missing links between self-replicating nucleic acids and the RNA world. Orig Life Evol Biosph 25:515–530
Johnston WK, Unrau PJ, Lawrence MS, Glasner ME, Bartel DP (2001) RNA-catalyzed RNA polymerization: accurate and general RNA-templated primer extension. Science 292:1319–1325
Joyce GF (2004) Directed evolution of nucleic acid enzymes. Ann Rev Biochem 73:791–836
Joyce GF, Orgel LE (2006) Progress toward understanding the origin of the RNA world. In: Gesteland RF, Cech TR, Atkins JF (eds) The RNA world, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 23–56
Kanavarioti A, Monnard P-A, Deamer DW (2001) Eutectic phases in ice facilitates nonenzymatic nucleic acid synthesis. Astrobiology 1:271–281
Kauffman SA (1986) Autocatalytic sets of proteins. J Theor Biol 119:1–24
Kauffman SA (1993) The origins of order: self-organization and selection in evolution. Oxford University Press, New York
Klussmann M, Blackmond DG et al (2006) Thermodynamic control of asymmetric amplification in amino acid catalysis. Nature 441:621–623
Lancet D, Shenhav B (2009) Compositional lipid protocells: reproduction without polynucleotides. In: Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) Protocells: bridging nonliving and living matter. MIT, Cambridge, pp 233–252
Lazcano A (2010) Historical development of the origins of life. In: Deamer DW, Szostak J (eds) Cold Spring Harbor perspectives in biology: the origins of life. Cold Spring Harbor Press, Cold Spring Harbor, pp 1–16
Lee DH, Cranja JR, Martinez JA, Severin K, Ghadiri MR (1996) A self-replicating peptide. Nature 382:525–528
Lee DH, Severin K, Yokobayashi Y, Ghadiri MR (1997) Emergence of symbiosis in peptide self-replication through a hypercyclic network. Nature 390:591–594
Lincoln TA, Joyce GF (2009) Self-sustained replication of an RNA enzyme. Science 323:1229–1232
Mansy SS, Szostak JW et al (2008) Template directed synthesis of a genetic polymer in a model protocell. Nature 454:122–125
Martin W, Russell MJ (2003) On the origin of cells: an hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Philos Trans R Soc London Ser B 358:27–85
Mavelli F, Ruiz-Mirazo K (2007) Question 8: bridging the gap between in silico and in vitro approaches to minimal cells. Orig Life Evol Biosph 37:455–458
Morowitz HJ (1992) Beginnings of cellular life. Yale University Press, New Haven
Morowitz HJ (1999) The theory of biochemical organization, metabolic pathways, and evolution. Complexity 4:39–53

New MH, Pohorille A (2000) An inherited efficiencies model of non-genomic evolution. Simulat Pract Theor 8:99–108
Nielsen PE (1993) Peptide nucleic acid (PNA): a model structure for the primordial genetic material. Orig Life Evol Biosph 23:323–327
Nielsen PE (2009) Peptide nucleic acids as prebiotic and abiotic genetic material. In: Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) Protocells: bridging nonliving and living matter. MIT, Cambridge, pp 336–346
Oparin AI (1953[1936]) Origin of Life. Margulis S (trans) Dover Publications, New York
Oparin AI (1965a) The pathways of the primary development of metabolism and artificial modeling of this development in coacervate drops. In: Fox SW (ed) The origin of prebiological systems and their matrices. Academic Press, New York, pp 331–341
Oparin AI (1965b) History of the subject matter of the conference. In: Fox SW (ed) The origin of prebiological systems and of their matrices. Academic Press, New York, pp 91–96
Orgel LE (1968) Evolution of the genetic apparatus. J Mol Biol 38:381–393
Orgel LE (2000) Self-organizing biochemical cycles. Proc Natl Acad Sci USA 97:12503–12507
Orgel LE (2003) Some consequences of the RNA world hypothesis. Orig Life Evol Biosph 33:211–218
Orgel LE (2004) Prebiotic chemistry and the origin of the RNA world. Crit Rev Biochem Mol Biol 39:99–123
Orgel LE (2006) Geothermal synthesis and metabolism. Astrobiology 6:297–298
Orgel LE (2008) The implausibility of metabolic cycles on the prebiotic earth. PLoS Biol 6:5–13
Paul N, Joyce GF (2002) A self-replicating ligase ribozyme. Proc Natl Acad Sci USA 99:12733–12740
Pino S, Cicieriello F, Costanzo G, Di Mauro E (2008) Nonenzymatic RNA ligation in water. J Biol Chem 283:36494–36502
Pohorille A (2009) Early ancestors of existing cells. In: Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) Protocells: bridging nonliving and living matter. MIT, Cambridge, pp 563–581
Powner MW, Gerland B, Sutherland JD (2009) Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature 459:239–242
Pross A (2004) Causation and the origin of life. Metabolism or replication first? Orig Life Evol Biosph 34:307–321
Rausch P, Savage D, Benner S (2004) UF study suggests life on earth sprang from borax minerals. http://news.ufl.edu/2004/01/08/earthstart/. Accessed 2 March 2010
Ricardo A, Carrigan MA, Olcott AN, Benner SA (2004) Borate minerals stabilize ribose. Science 303:196
Ross DS (2008) A quantitative evaluation of the iron-sulfur world and its relevance to life's origin. Astrobiology 8:267–272
Russell MJ, Hall AJ (1997) The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. J Geol Soc Lond 154:377–402
Russell MJ, Martin W (2004) The rocky roots of the acetyl-CoA pathway. Trends Biochem Sci 29:358–363
Segré D, Ben-Eli D, Deamer DW, Lancet D (2001) The lipid world. Orig Life Evol Biosph 31:119–145
Shapiro R (2007) A simpler origin of life. Sci Am 296:47–53
Shenhav B, Segré D, Lancet D (2003) Mesobiotic emergence: molecular and ensemble complexity in early evolution. Adv Complex Syst 6:15–35
Smith E, Morowitz HJ (2004) Universality in intermediary metabolism. Proc Natl Acad Sci USA 101:13168–13173
Smith E, Morowitz HJ, Copley SD (2009) Core metabolism as a self-organized system. In: Rasmussen S, Bedau MA, Chen L, Deamer D, Krakauer DC, Packard NH, Stadler PF (eds) Protocells: bridging nonliving and living matter. MIT, Cambridge, pp 433–460
Smith JM (1998) Shaping life: genes, embryos and evolution. Weidenfelf & Nicolson, London
Sterelny K, Griffiths PE (1999) Sex and death. The University of Chicago Press, Chicago
Szostak JW (2009) Systems chemistry on early earth. Nature 459:171–172
Szostak JW, Bartel DP, Luisi PL (2001) Synthesizing life. Nature 409:387–390
Troland LT (1914) The chemical origin and regulation of life. Monist 24:92–133
Wächtershäuser G (1988) Before enzymes and templates: theory of surface metabolism. Microbiol Rev 52:452–484
Wächtershäuser G (1992) Groundwork for an evolutionary biochemistry: the iron-sulphur world. Prog Biophys Molec Biol 58:85–201
Wächtershäuser G (1994) Life in a ligand sphere. Proc Natl Acad Sci USA 91:4283–4287
Wächtershäuser G (2007) On the chemistry and evolution of the pioneer organism. Chem Biodiv 4:584–602
Whitfield J (2009) Origin of life: nascence man. Nature 459:316–319
Zaher HS, Unrau PJ (2007) Selection of an improved RNA polymerase ribozyme with superior extension and fidelity. RNA 13:1017–1026