The non-genetic dawn of life

a review of the contemporary standing of the metabolism-first approach to the origin of life enigma

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5 May 2013

A master thesis submitted to Universiteit Utrecht, Environmental Biology

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SUMMARY: the origin of life on early Earth is unresolved still. Two approaches to the problem dominate the debate. Replicator-first proponents search for the very first replicator molecule. A much envisioned molecule in this regard is RNA. Metabolism-first adherents deem it unlikely that a replicator molecule emerged *de novo* on early Earth, and instead search for a network of small molecules that could have seeded modern life. Three metabolism-first scenarios are discussed. The elaborate lipid world scenario proclaims the Darwinian selection of early metabolic networks. It is shown that even though persisting entities may have come into existence, heredity in ensemble replicators is probably too inaccurate to allow for their selection, and even if accurate enough inherently attractor based, posing severe problems for their subsequent evolution. The problems are exemplary for scenarios that pose the evolution of metabolic networks. The only demonstration to date of the accumulation of novel mutations in ensemble replicators is provided in a far-off from real chemistry polymer model. The evolutionary dynamics encountered in the model depend heavily on the combinatorial chemistry of polymers, and cannot be generalised with the same success to monomer models. Moreover, many open questions remain. It is concluded that the standing of Darwinian selection in metabolism-first scenarios is problematic. The proposition that life started with the reverse tricarboxylic acid cycle that arose due to geochemical ordering is discussed as an example of a preparatory metabolism theory. As yet, it has not been shown that the cycle arises spontaneously in geothermal environments. The repertoire of catalysis by small organic molecules and mineral surfaces seems to be a crucial factor. A thermodynamic framework for non-equilibrium systems is lacking. However, experiments in hydrothermal reactors might provide the answer: the possibility that part of metabolic complexity is merely the result of thermodynamic ordering makes such experiments worthwhile the effort. It is concluded that the claim that metabolism-first theories provide a robust alternative for replicator-first scenarios cannot be supported yet based on the three hypotheses. Questions posed in the present paper may provide guidance for research to come.

1. Introduction

The emergence of life on early Earth is as yet ill-understood. Ever after the ubiquitous abiogenic synthesis of organic molecules (Follmann and Brownson, 2009), complex life arose as an emergent property of its constituents, of which the oldest evidence, stromatolites and microfossils, dates back to 3,5 billion years ago (Schopf *et al.*, 2007). However, the series of steps that leads from mere organic chemistry in early Earth conditions to biology is unsolved still. The quest for the emergence of life on Earth is complicated further by the retrospective constraint that any imagined prebiotic entity must have given rise to the staggeringly diverse yet also restricted complex biotic realm of today. An answer to the question how contemporary life began must thus deal with both bottom-up and top-down limitations (Forterre and Gribaldo, 2007), making the search extremely difficult and the various approaches numerous.

The present paper reviews the state of the art of the diverse origin of life research field, with a focus on one prevalent hypothesis:

the first important step towards modern life was the onset of metabolism, instead of the emergence of an informational molecule.

Its aim is to identify most promising directions for studies to come. After an introduction into the field, three extant metabolism-first scenarios are discussed, resulting in an answer to the main question: how far has metabolism-first thinking hitherto come in an explanation for the origin of life?

Breakdown of the question

The question of the emergence of life on Earth represents a subset of the more general question: how to derive very complex systems from simpler ones? Chemical as well as informational challenges accompany the question. As has been clearly put forward by others (e.g. Dawkins, 1986), cumulative selection of single-step small chance events is the only known viable explanation for anything as complex as modern life, i.e. a persistent existence of the highly improbable. However, any evolvable system had to get started in the first place. Part of the answer must be (self-)organisation of matter, i.e. the build-up of order out of chaos, prior to natural selection. Moreover, if an evolvable system did fall into place, Darwinian selection need not necessarily have resulted in ever-higher complexity, as was demonstrated by Spiegelman's monster: an RNA replicase that under constant selection pressure reduced its genome size to an absolute minimum (Oehlenschläger and Eigen, 1997). In addition, the discussion of the error threshold and the problem of parasites further on shows that evolution towards higher complexity is far from evident.

The focus in origin of life research therefore lies on chemical (self-)organisation (i.e. what kind of chemical systems are (self-)organising?), the synthesis of primitive evolvable systems (i.e. how do (self-)organising systems lead to evolvable systems?), and the subsequent evolution of such a system (i.e. how does evolution of the system results in higher complexity?). Eventually, a conclusive explanation for the origin of life should account for all three questions, within the boundaries given by the top-down constraints of modern biochemistry and bottom-up constraints of early Earth environmental conditions.

Two hypotheses for the very origin of life

The scientific debate on the origin of life has historically been divided into two schools of thought that, each in their own way, deal with these questions: the *replicator-first* and *metabolism-first* scenarios (Anet, 2004). The schools disagree, i.e. propose different hypotheses, on what came first in life's history: an autocatalytic molecule (resembling contemporary informational molecules like DNA) or a sequence of chemical reactions that as a whole was autocatalytic (resembling contemporary metabolism). The key question is, in other words: did life start as a string of covalently bonded, or as a set of non-covalently bonded molecules? The theories have in common the necessity of autocatalysis, which allows for initial order to persist and propagate.

No school has a conclusive explanation for the emergence of life yet (note however, that no school pretends this either: they merely propose the starting point of the path towards complex life). Smaller questions that investigate part of the problem are and have been of importance for the two hypotheses. Because metabolism- and replicator-first scenarios face distinct initial challenges, the schools have commenced from different directions to falsify their theories: most metabolism-first thinking has been concerned with the question how higher levels of complexity can be reached by principles of self-organisation; replicator-first thinking has more strongly been concerned with the question how, given a replicator system, higher levels of complexity can be reached by natural selection.

While this notion clearly oversimplifies the debate, it does illustrate the fragmentation of the field concerning the questions that are asked. An 'explanation for the origin of life' might in scientific literature mean different things to different authors. Also, it is apparent that deciding whether metabolism or a replicator came first in the emergence of life is a naive quest that leads nowhere, if not first all three above-stated questions have been addressed: even though the two schools do not claim a conclusive answer to the emergence of life, eventually they must have one in order to deem the hypothesis plausible. Identifying the unanswered questions in both scenarios is for the time being a much more promising approach. In other words: deciding what needs explanation for the origin of life

is the best that can be done to get to an eventual answer; a notion that virtually summarises the state of the art of the research field (see e.g. Luisi and Ruiz-Mirazo, 2009; Rain and Luisi, 2012; as well as their accompanying papers).

The schools can hence be viewed as two different approaches to the same problem, instead of two competing scenarios for the origin of life. Such a view also allows for the possibility that metabolism and informational molecules arose independently and only later merged.

The present paper reviews metabolism-first scenarios in the contemporary scientific debate. Knowing that a conclusive answer cannot be given yet, the question posed in this paper is: did life on Earth start as a metabolic network? In an attempt to answer the question, gaps in knowledge that make the question as yet unanswerable are identified, exposing fruitful directions for origin of life research to come.

2. The competing hypothesis: replicator-first and the RNA world

One cannot discuss metabolism-first without mentioning the alternative scenario. Partly because many workers in the field nowadays adhere to a replicator-first scenario, in which the role of the first replicator was fulfilled by an RNA molecule. But especially because serious objections to the spontaneous emergence of this self-replicating RNA molecule (or indeed any self-replicating molecule, considering that such a molecule must likely be fairly complex already) have spurred, or rather kept alive, the metabolism-first hypothesis (see e.g. Shapiro, 2000); in origin of life research, the improbability of one hypothesis is often considered as support for the other. Even though such an approach seems naive, it has historically been a driver of the debate (not without success, and should one scenario turn out to be inherently impossible, the least that can be said is that the alternatives seem more likely), and a short discussion of the contemporary standing of the replicator-first scenario with a justified emphasis on the RNA world will illuminate the metabolism-first scenarios to come.

A top-down explanation for the origin of life: RNA-first

The spontaneous emergence of a self-replicating polymer from a diverse soup of complex organic molecules marked the beginning of natural selection and the starting point on the pathway to life in the replicator-first scenario. The hypothesis is appealing because the molecule itself is both catalytic and possibly has the ability to pass on information, its chemical identity, to next generations. Since the two are directly related in the model, the replicator-first scenario represents the simplest evolvable system in a mathematical sense; the replicator is the most basal unit of evolution (*sensu* Zachar and Szathmáry, 2010). Even more so, the scenario reminds us of the template-dependent replication of DNA molecules that is utterly important in the contemporary biosphere.

The hypothesis is an answer to the top-down motivated question: what is the simplest system we could think of that could lead to modern life? By simplifying living systems, one arrives at the abstract replicator. (Note that asking a top-down motivated question differs from a top-down approach to the question, namely directing a system towards modern life; the here imagined entity can still be subjected to non-goal directed modelling, and the question that should be addressed then is how the system evolves to something as complex as modern life.) If the question can be answered with realistic chemistry, an explanation for the origin of life might be near. Proponents feel that no other processes are needed to resolve the origin of life enigma.

The most frequently mentioned molecule in this regard is RNA. Since the discovery that some RNA molecules mediate self-splicing and thus act as enzymes (appropriately called Ribozymes; Kruger *et al.*, 1982), the recognised repertoire of RNA catalysis has been greatly enlarged (Cech, 2002; Doudna and Cech, 2002). It is now widely accepted that RNA can both serve as information carrier and catalyst, and is thus potentially an answer to the "Chicken-and-egg" paradox of the origin of life: DNA is constructed by proteins and proteins are coded for by DNA, so which came first? RNA could be both

the chicken and the egg (Bernhardt, 2012).

Consequently, many have come to believe that life started from an RNA-dominated stage, the RNA world (Gilbert, 1986; Joyce, 2002; as already imagined earlier by e.g. Crick, 1968), in which at some point RNA molecules catalysed their own synthesis. The ability of the molecules to pass on acquired mutations (any change in the molecule that alters the information stored while keeping its ability to replicate intact) due to template-dependent replication and therefore experience heredity, made the RNA world subject to natural selection and evolution (as has been demonstrated in controlled conditions (Beadry and Joyce, 1992; Joyce, 2004)), resulting in increasing molecular complexity; an assumption that need not necessarily be true, but instead must be seen as the hypothesis to test. Only later on, after RNA evolved properties to catalyse peptide and amino acid polymerisation, did the familiar proteins and DNA take over most of catalysis and all information storage, respectively.

In summary: the RNA-first hypothesis is tempting from a top-down perspective on the emergence of life, due to the supposed continuity between the very origin of life and modern biochemistry. As such, it has become a much envisioned scenario.

However, serious challenges to the scenario have led some researchers to (or kept them with) other propositions for the starting point of life: either a different replicator molecule, or the dismissal of an informational molecule as the starting point of life altogether in metabolism-first scenarios.

Challenges for the RNA-first hypothesis

The RNA-first scenario lacks the ultimate experimental evidence. As early as 1975, it was shown that truly self-replicating RNA structures could emerge, however only in the presence of the complex Qβ replicase enzyme (Sumper and Luce, 1975). Recently, an RNA-only self-replicating RNA system was demonstrated to exist, however composed of two ribozymes that cross-catalyse each others synthesis from four component substrate molecules (Lincoln and Joyce, 2009); even though close to, not yet the dreamed-of molecule.

Also, assuming that a single RNA replicator did emerge, difficulties in maintaining beneficial information arise when the system evolves and strings become longer, because of the mutation dependent error threshold (Eigen, 1971; Eigen, 2000; for a recent discussion see Takeuchi and Hogeweg, 2012); a concept that was discovered *in silicio* but has proven invaluable for the understanding of real life evolutionary dynamics (see e.g. Lauring and Andino, 2010). Obviously, in a pre-enzymatic age mutation rate would have been high, resulting in short maintainable string lengths; possibly too short for any notable mutation rate reducing catalytic function. Progress has been made in finding viable solutions for the paradox (Eigen and Schuster, 1977, 1978; Zintzaras *et al.*, 2002; Kun *et al.*, 2005; Takeuchi *et al.*, 2005; De Boer and Hogeweg, 2010; Vaidya *et al.*, 2012), but as for now a definitive answer has not been given. Moreover, the first combined replicator systems would have faced the problem of the invasion of parasites: molecules that benefit from the replicator system but do not aid in replication of the system itself (namely non-catalytic mutants; also 'short-circuit' parasites would have been a problem). The result is the likely destruction of the system, i.e. selection towards a dead end instead of the sought higher complexity. The only way out is through the fulfilment of certain conditions, e.g. compartmentalisation (Boerelijst and Hogeweg, 1991; Szabó *et al.*, 2002; Takeuchi and Hogeweg, 2007; Bansho *et al.*, 2012).

While these informational challenges have been addressed in RNA-like systems and do pose major problems for the evolution towards complexity in the replicator-first scenario, their presence in the replicator-first camp primarily reflects that a central question for replicator-first proponents has been how natural selection leads to higher complexity and eventually to modern biochemistry, as well as the near absence of the question in the metabolism-first camp. Any unit of evolution that on its evolutionary path increased the complexity of its genome must likely have faced and overcome hurdles, no matter what the chemistry of the system or how it started: as an autocatalytic molecule or as an autocatalytic set of chemical reactions (although the nature of the problems might differ per approach due to the different dynamics; e.g. ensemble replicators face severe issues regarding preservation of information over generations as will be discussed later, yet at the same time are not readily destroyed by parasites (for a discussion of the effect of side reactions on ensemble replicators, see Szathmáry, 2000)).

So even though evolvability issues have surfaced mainly in the replicator-first camp with

RNA-like replicators, their presence is of importance to the entire origin of life research field, and (not) solving them does hardly make any of the hypotheses stand stronger. In fact, evolvability issues might pose an even more severe problem for ensemble replicators (in the sense that evolution of ensemble replicators might not be possible at all), as will become apparent later. These problems are thus certainly a challenge for the RNA-first scenario, yet at the moment not conclusive for a rejection of the replicator-first (either RNA or a different molecule) hypothesis.

Not surprisingly, objections to the RNA-first hypothesis that do favour other scenarios must be sought in the very beginning, and they stem mainly from a bottom-up perspective on the origin of life.

The most serious challenge that the RNA-first scenario faces is that a self-replicating RNA molecule, even though mathematically simple and elegant, might be chemically too complex to have arisen by mere prebiotic chemistry (Joyce, 1989). There are infinite more ways to combine atoms in other arrangements than in an RNA molecule (let alone one that is able to replicate itself), and the thermodynamic laws that bias the odds do not seem to favour an RNA world in the supposed environmental conditions of early Earth (Shapiro, 1984; Joyce, 2002; Orgel, 2004; Anastasi *et al.*, 2007). In addition, RNA is unstable in any but a few rare environmental conditions (Bernhardt, 2012). In particular because self-replicating RNA molecules rely on the steady supply of (clean; but see Trevino *et al.*, 2011) (oligo)nucleotides, the amount of luck that seems necessary to deem the scenario plausible appears problematic high, even still in the light of the recently discovered mechanism of relatively stable pyrimidine ribonucleotide synthesis in 'prebiotically plausible conditions' (Powner, *et al.*, 2009); a reaction sequence that 'must be judged as an extremely unlikely representation of geochemical events before life began' (Shapiro, 2010, p. 424). The gap between prebiotic chemistry and a fully fledged RNA world lingers.

In the words of Pier Luisi, the RNA-first scenario 'opens more problems that [sic] it is capable of solving' (Luisi, 2012, p. 2636). As such, a small RNA molecule that arose *de novo* and seeded modern life is considered a myth by some (Robertson and Joyce, 2012).

Different approaches to the improbability of RNA-first

Many scholars nowadays include the RNA world at some point in their theories, but disagree on the order of events (Robertson and Joyce, 2012). Largely as an answer to the seemingly unlikely emergence of a *de novo* RNA world, present-day scientific literature dealing with the question 'what came first' is focused around three directions of research (the reader may remark that some of the following hypotheses have come about without reference to the RNA world or the RNA-first scenario; while this is true, the idea is at present so firmly established that one can hardly deny that it is the scenario to beat, and the burden of proof lies first with the opposing scenarios):

1. *RNA-first*: a search for as yet unknown chemical mechanisms that make a *de novo* RNA world thermodynamically plausible on early Earth conditions, either by pursuit of new chemical pathways or a new sequence of events (Ma *et al.*, 2007; Briones *et al.*, 2009), and/or by redefining the early Earth environment (as summarised by Jortner, 2006). The reader is referred to metal-ion catalysed synthesis of oligomers, e.g. in montmorillonite clay environments (Ferris, 2006; Huang and Ferris, 2006), as a most promising direction of research for the origin of the RNA world. Also, hydrothermal vent systems provide ample suitable environments for almost any origin of life theory, including RNA-first (Baaske *et al.*, 2007). Recently, the possibility of an extraterrestrial origin for nucleobases has again gained some support (Callahan *et al.*, 2011), in line with the ever-growing evidence that complex organic molecules are formed in outer-space (see e.g. Jørgensen *et al.*, 2012).
2. *Replicator-first but not RNA-first*: the dismissal of RNA as the first autocatalytic molecule and the search for another one, e.g. 'simpler than RNA' structures such as threose nucleic acid (Orgel, 2000; Yu *et al.*, 2012; but see Anastasi *et al.*, 2007), roughly equally complex molecules such as proteins (Kurland, 2010) or an even more complex protein/RNA combination (Caetano-Anollés *et al.*, 2011; Harish and Caetano-Anollés, 2012; although the kind of evidence that is postulated, phylogenomic analyses, seems inconclusive for a world that left no traces in contemporary genomes (Bernhardt, 2012); also, the polymers need not necessarily be

replicators but can be envisioned in a metabolism-first scenario, see further on and e.g. Egel, 2009). Indeed the latter scenario further complicates the RNA-first scenario, but it can just be so that there is even more that needs explanation for a viable solution to the origin of life enigma. The more exotic idea of an inorganic crystal replicator still surfaces in recent literature (Bullard *et al.*, 2007; Cairns-Smith, 2008), but especially the huge gab between the replicator and contemporary biochemistry has resulted in sparse support for the scenario. A later stage in these replicator-first scenarios may or may not encompass the RNA world ('RNA-later').

3. *Metabolism-first*: the dismissal of any informational molecule as the first important chemistry in the emergence life, and the search for a metabolic network of small or medium sized molecules that could have seeded modern life. The RNA world may or may not have been part of the scenario at some point. The crucial assumption is that life started as a set of fairly simple interacting molecules, thereby overcoming the problem of the much needed initial molecular complexity in the replicator-first scenario (Shapiro, 2000). Any subsequent step, be it an RNA world or something else, originated from the already self-assembled, possibly evolved, system or from an environment that was shaped by it.

The remainder of this paper is concerned with (3) as opposed to the replicator-first scenarios of (1) and (2). The appeal of a metabolism-first scenario for the origin of life lies in the seemingly less complexity (in other words: the lower dependence on a single very unlikely event) that is needed to get life started; the superficial notion should not be regarded as an argument for the hypothesis but rather as an observation that warrants further investigation. Because metabolism-first theories represent mainly a bottom-up perspective on the origin of life (i.e. they deal with the question: given early Earth conditions, how does one get to complex life?), their explanations can yield crucial insights on a fundamentally different level than that attained by replicator-first scenarios. Also, top-down motivated, it is an arousing question whether metabolic networks could have arisen and seeded modern life before the existence of an informational molecule that exerted its control.

At this point, it is worth noting that a select group of theoretical biologists is working on models that encompass both replicator molecules and metabolism, thereby trying to bridge the gap between the replicator- and metabolism-first scenario. Note that metabolism and an informational molecule must have arisen together at some point in the history of life, but that their partnership need not necessarily had to be established already in the very beginning, so bridging the gap is not a requisite for an explanation to the origin of life, but rather another hypothesis. The reader is referred to the work of Szathmáry and coworkers (Könnyű *et al.*, 2008) in particular, in which the replication of short macromolecules is being fuelled by the production of monomers from a metabolic network. These attempts are interesting, albeit as yet not extremely fruitful, and lie beyond the scope of this paper.

From hereon, contemporary scenarios for a non-genetic origin of life are reviewed.

3. Metabolism-first

Scenarios in which the first entities on the pathway to life were loose, non-covalently bonded, molecular assemblies have been put forward numerous times in origin of life research (Farmer *et al.*, 1986; Morowitz, 1992; Kauffman, 1993; Dyson, 1999; Segré *et al.*, 1998a; see also Anet, 2004). They are modern variants of the abandoned coacervate hypothesis of Alexander Oparin (Oparin, 1938; he already proposed the scenario 14 years earlier, however because it was written in Russian his theory received little attention at the time (Miller *et al.*, 1997)), who constructed his ideas in an age before the discovery of the molecular structure of DNA (and RNA), and hence any replicator-first scenario.

They are nowadays referred to as metabolism-first scenarios for the origin of life. As Dyson already noted (Dyson, 1999), metabolism is a vague concept compared to the clear notion of a replicator in replicator-first theories. But precisely the contrast with replicator-first nowadays defines metabolism-first. Proponents of metabolism-first theories dismiss replicator-first theories and search

for a scenario in which no single informational molecule was in charge. Instead, they ask: could the emergence of a network of simple molecules be the first important step in the origin of life?

Metabolism-first is thus the collective term for any theory that aims to answer this question, and encompasses both scenarios that use the term in a close to contemporary meaning of the word metabolism (i.e. part of metabolism in modern organisms is the most ancient attribute of life), as well as scenarios that use it in the broad meaning of the expression (i.e. life started as an autocatalytic set of (small) molecules, not necessarily resembling metabolism in modern organisms). Note that the hypercycle is autocatalytic as a whole but differs from metabolic networks in metabolism-first scenarios, in that in the latter individual molecules are not replicators themselves.

Also, opinions differ on the role of the first metabolic networks in the origin of life, especially regarding the onset of natural selection. Iris Fry (Fry, 2011) has coined the term 'metabolism-first' for any theory that postulates that the first entities subject to Darwinian selection were metabolic networks (note that this nomenclature is not followed in the present paper), and preparatory-metabolism for any metabolism-first theory that regards the onset of natural selection as the domain of replicator molecules. As an example of the latter, Christian de Duve has stressed the necessity of 'protometabolism' for the origin of life (De Duve, 2003), yet acknowledged the central role of a replicator molecule in the onset of evolution (De Duve, 2005).

The following aims to summarise the progress made by three extant theories, whose discussion in scientific literature can to a large extent be regarded as the state of the art of the metabolism-first scenario. The standing of Doron Lancet's lipid world theory for the origin of life illuminates the problems encountered by theories that proclaim metabolic networks to be the first Darwinian entities. A short detour is dedicated to a recently published thought that might provide a way to overcome some problems. Eric Smith's bold ideas of life emerging as a necessity via metabolism represent a far-reaching preparatory-metabolism theory as well as an intriguing attempt to bridge the gap living and non-living matter and between physics and biology. By no means are these three propositions the only metabolism-first theories around, however some of their conclusions can be generalised. An attempt is made to do so whenever possible.

3.1 The lipid world

Doron Lancet and coworkers envisage a lipid world in which life took its first steps (Segré *et al.*, 2001a; Shenhav *et al.*, 2003; Bar-Even *et al.*, 2004; Segrè and Lancet, 2005): the self-assembly of amphiphilic compounds (from hereon called lipids, contrary to everyday language in which the term lipid is reserved for a subset of amphiphilic compounds) into 'mesobiotic composomes', their 'ensemble replication', and the subsequent evolution of these composomes. The lipid world gave rise to a system in which far more complex molecules had taken over as information carrier and the lipid's role was reduced to its contemporary membrane function. It is possible that somewhere along this path to higher complexity the RNA world arose (see Segré *et al.*, 2000b), although the lipid world hypothesis is not necessarily concerned with the transition to an RNA world; the focus for now lies on the very first steps of the scenario. The important claim the theory makes is that what came before the first replicator molecule was already capable of some rudimentary life-like dynamics, among which the ability of the lipid ensembles to undergo evolution. In other words: the theory states that Darwinian selection can act on populations of molecules without genetic apparatus, resulting in evolution towards higher complexity. The lipid world scenario is hence regarded by many as an archetypal metabolism-first scenario.

Lipid entities remind us of contemporary cellular membranes, and thus do not appear too unlikely as a starting-point for life, at least superficially: any pathway towards complex life had to incorporate lipid membranes at some point, so why not start with the lipids? Also, self-assembly of lipids into aggregates (vesicles or droplets) is readily achieved in aqueous environments. Order is thus cheap, for the lipids tend to order themselves; the concentration mechanism comes for free.

The molecules themselves are easily formed in an organic soup, considering that almost any long hydrocarbon with a polar group is amphiphilic. Therefore, at least the formation of vesicles can be considered a general event, in sharp contrast to the spontaneous formation of an RNA molecule; the most remarkable evidence perhaps being the emergence of lipid droplets when organic compounds extracted from the Murchison meteorite were exposed to water (Deamer and Pashley, 1989; incidentally the same meteorite on which later traces of nucleobases were found (Callahan *et al.*, 2011). The key question is: can such vesicles be subjected to Darwinian selection?

A priori assumptions

The lipid world scenario rests on a few *a priori* assumptions. The most important are:

1. Early Earth conditions favour the formation of an organic soup in an aqueous environment that contains a myriad of different molecules, among which a great variety of lipids that tend to aggregate because of a free energy difference between aggregated and solitary molecules. In the scenario, no real vesicles are imagined but rather clumps of lipids, or ensembles. It is not clear what is exactly meant by this, but it suffices to think of vesicles or droplets in which the interior fulfils no function: all involved molecules and the vesicle are the same entity. From hereon, the term vesicle is used for these aggregated lipids.
2. Various molecules in the soup can exert at least some catalytic power, lipids included (for examples of lipid catalysis see Klijn and Engberts, 2005; Zepik *et al.*, 2007). The incorporation of lipids from the environment into a vesicle (namely into the ensemble, not into the interior, for as described above interior is devoid of meaning in this context) as well as the secretion of lipids out of the vesicle may also be subjected to catalytic rate enhancement by lipids already present in the vesicle. The extent of catalysis depends on the molecules present as well as on their concentration. Note that the term catalysis is used in the abstract sense as 'any process that enhances a rate', and chemical mechanisms underlying it are not further specified.
3. Lipid vesicles will split when a certain size is reached; thereby ensuring 'progeny' of the vesicle as well as a mean of keeping the system away from equilibrium.

Especially (2) is questionable. However, the above-stated assumptions are for the moment deemed authentic, in order to investigate the dynamics of the proposed scenario. Later on the role of (2) in the scenario is discussed.

The scenario: compositional genomes

The lipid world scenario presumes that, given the above-stated assumptions, an early Earth-like environment contains an organic soup in which a great many of different lipid vesicles are present. The difference between vesicles comes about by a difference in the combination of lipids that make up an assembly, given that any vesicle contains a restricted set of monomers as compared to the total variation of monomers present in the environment.

Most of the vesicles will do something completely random, i.e. they do not persist in time. However, some have a compositional make up that ensures survival. In these special cases, every molecule catalyses the incorporation in the vesicle of another molecule that is already present, in such a way that the entire ensemble is autocatalytic (note that even though the molecules in the vesicle 'self-replicate', or better: self-propagate, by incorporation of molecules from the environment, the entire vesicle at this point does not). The result is 'homeostatic growth', meaning that the vesicle grows while its composition stays the same.

The composition inherently contains information: an oligomer out of 20 different monomers with a length of 23 contains roughly as much information as a vesicle that contains between 1 and 20 copies of each of 23 types of monomers (while the amount of required monomers may not be the same, the order of magnitude is comparable in the two scenarios (see Shenhav *et al.*, 2003), at least as long as a small number of molecules is concerned (see however Szatmáry, 2006 and further on for a discussion on the upper bound of information that can be stored)). The persistent vesicles therefore are said to have compositional genomes, or composomes (for a general discussion of composomes, see Hunding *et*

al., 2006). When a vesicle splits, (part of) its contained information can be transferred to progeny. Even if the fission is completely random, chances are the progeny will look alike, i.e. their compositional information is the same as the parent vesicle, especially if the number of different molecules in the parent is a lot smaller than the absolute number of molecules (note that if every molecule is the same, information transfer becomes trivial). In other words: inheritance is a property of the system, and so are 'mutations', considering that transmission of information is not accurate in every instance. Every splitting process is hence comparable to the replication of a replicator molecule, and the vesicles are subjected to selection and evolution as well, the scenario states. It is these vesicles that are hypothesised to be the earliest ancestors of modern life.

It is doubtful whether the lipid world scenario will ever be subjected to wet experiments. Dynamics of the scenario have thus far only been studied *in silicio* (an approach that according to Lancet and coworkers might become the rule in origin of life research (Shenhav and Lancet, 2004)), using a quantitative mathematical model: the Graded Autocatalysis Replication Domain model, or GARD.

GARD dynamics

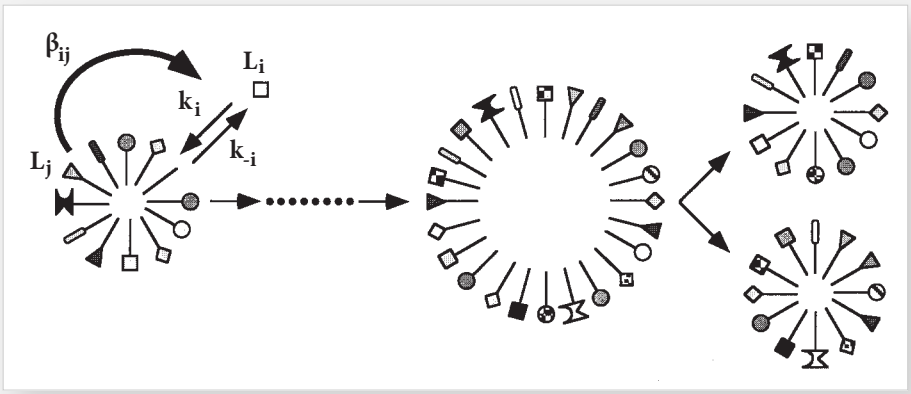
GARD provides a quantitative analysis of self-replicating molecular ensembles in mutually catalytic sets based on chemical kinetics. Different variants have been introduced (summarised by Segrè and Lancet, 2005; see also Shenhav *et al.*, 2005; Shenhav *et al.*, 2007, Markovitch *et al.*, 2012). The so-called Amphiphile-GARD or A-GARD is the most applicable for the lipid world scenario.

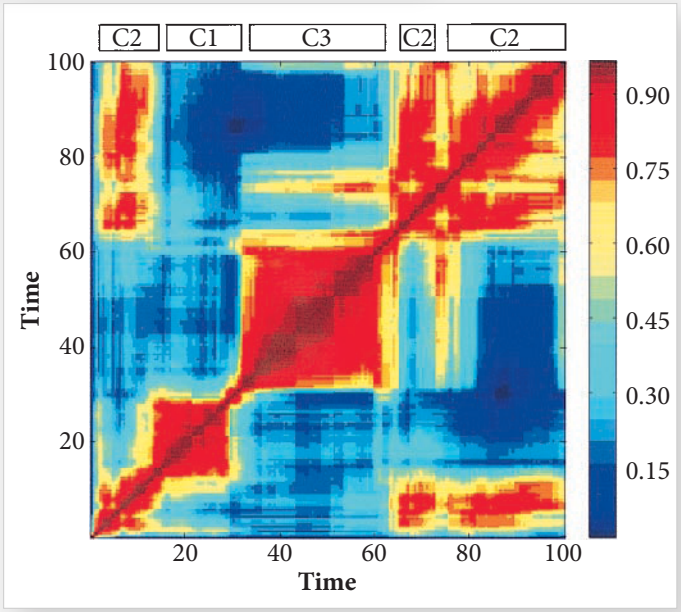
The GARD model (Segré *et al.*, 1998a; Segré *et al.*, 1998b) consists of differential equations that describe the incorporation and secretion of molecules into and out of a vesicle (any restricted spatial domain; a lipid-like vesicle is readily imagined), whose rates are enhanced (i.e. the reactions are catalysed) by molecules that are already present in the vesicle, over time (Figure 1A). In the A-GARD model, the vesicle and molecules are the same entity. Only molecules that exert catalysis are concerned, and their catalytic power is determined by the β matrix: a N_G x N_G positive matrix in which N_G is the total of different environmentally available molecules. The values of the matrix are randomly drawn from a log-normal distribution: few molecules exert very strong catalysis while most exert only weak catalysis. Catalysis is carried out if molecules are part of the vesicle, in a concentration-dependent manner.

In an ordinary simulation, a vesicle is followed over time, given a random initial composition of the vesicle (of size N_{min}) and a supply of food molecules from the environment. The total of molecular types in the vesicle (N_E) is kept smaller than N_G ($N_E << N_G$; if not, the dynamics of the system become trivial and very boring). Mostly, the total number of molecules in the universe (environment + vesicle) is kept constant. A population of GARDs can also be followed over time, given a restricted amount of shared food molecules, hence reflecting dynamics in a competitive environment.

It has been shown that a vesicle inevitably decays to equilibrium conditions if it is allowed to grow in a closed system (Segré *et al.*, 2000a), i.e. with a finite supply of food molecules, which can be regarded as rather trivial. However, if the vesicle is allowed to split in equal halves if it reaches a threshold size ($2N_{min}$; one of the progeny assemblies is thereafter randomly chosen to be decomposed, and the molecules in the environment are replenished), thereby disrupting the process and keeping the system away from equilibrium, much more interesting dynamics arise: the system now turns to a quasi-stationary-state, in which compositional information is preserved (Figure 1B). Multiple such attractors exist in composition space, which are called composomes (clusters of highly related composomes are called compotypes, and perhaps better resemble the actual quasi-stationary-state, considering that within a compotype turnover (flipping) of composomes is readily achieved; from hereon, the designation composome is primarily used for the quasi-stationary-state and can thus also refer to compotype). A composome arises because on this particular point the entire composition is autocatalytic (i.e. closed mutually catalytic); once a vesicle gets there, homeostatic growth ensures that the composition is retained within a generation. The states are stable for time periods that encompass multiple generations, due to the rudimentary inheritance of the vesicles.

A composome might therefor be the persistent existence of order that is sought for in the explanation for the emergence of life: the entity that can undergo Darwinian selection.





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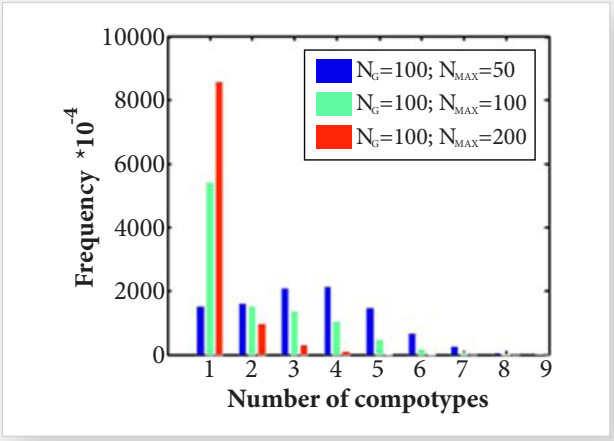


Figure 1. The GARD model describes catalysed growth of a lipid vesicle (A). L_j and L_i are different amphiphilic species. k_i and k_{-1} are spontaneous incorporation and secretion rates, that are enhanced by molecules already present in the vesicle, according to the randomly assigned log-normal distributed β matrix. The number of different environmentally available molecules (N_G) is defined from beforehand. If the vesicle goes through a growth-splitting process, non-trivial behaviour can emerge. Quasi-stationary-states in composition space are evident in GARD similarity carpets (B), that depict the time-correlation matrix of the similarity parameter *H*. In the simulation in (B), N_G=100, the total number of molecules in the universe (environment + vesicle) is 1000, and N_{min}=40. Logarithms of catalytic rate enhancement values are derived from a normal distribution with μ=-4 and σ=4. Quasi-stationary-states are called compotypes (C1, C2, and C3 in (B)). Due to stochastic splitting of the vesicle, abrupt transitions from one compotype to another may occur. The number of compotypes in a simulation depends on the initial composition of a vesicle, but also on the pre-fission assembly size (N_{max}) relative to N_G (C): the smaller the vesicle before splitting, the higher the compotype diversity. Figures from Segré *et al.*, 2001a (A), Segré *et al.*, 2000a (B), Markovitch and Lancet, 2012 (C).

Two remarks must be made.

First, the nature, number, and existence at all of composomes depends on a lot of factors, including kinetic parameters (both basal kinetic rates and enhanced kinetic rates; a higher average catalytic value results in higher parent-progeny similarity and more stable composomes that contain more information (Segré *et al.*, 2000a)), assembly size (there seems to be an optimal assembly size related to N_G (Segré *et al.*, 2001b)), and extent of mutual catalysis as opposed to autocatalysis (Markovitch and Lancet, 2012), but above all on the distribution of the catalytic rate enhancement values, i.e. on the β matrix: any deviation from a log-normal distribution will result in a system without

stable composomes, because the transfer of information to next generations becomes impossible due to frequently occurring 'compositional error catastrophes' (comparable to the error catastrophe in the RNA-first scenario; Segré *et al.*, 2001b). Only a log-normal distribution (and, depending on the other parameters, not even every log-normal distribution) will ensure a high enough reproduction fidelity. Especially because the chemical mechanisms of the imagined catalysis are not specified, nor demonstrated in the laboratory, it is difficult to assess whether such a distribution is realistic (the log-normal distribution is based on the receptor affinity distribution which finds its origin in a drug pharmacological type set of experiments, see Segré *et al.*, 2001b). So, even *if* the early Earth environment contained solely catalytic lipids (which of course it did not; one could argue that composomes filter out noisy environmental inputs so it does not matter whether there are also other molecules around, however such an argument does not hold for distinct chemical reactions, and it has not been shown in a spatial manner either), composomes emerged only if the conditions were appropriate. As opposed to the emergence of lipid vesicles, a quite special early Earth environment is needed for the emergence of composomes.

Second, because of the stochastic splitting of vesicles, abrupt transitions from one composome to another may occur. A system therefore generally passes through a number of quasi-stationary-states. Most publications are not explicitly dealing with the lifetime of composomes, but judging from the so-called compositional correlation carpets that mainly show absolute time correlations, quasi-stationary-states do not appear to span long periods of time and thus many generations: e.g. in Segré *et al.*, 2000a the most stable composomes survive for about 20 time steps (further on, the lifetime of composomes is more elaborately discussed). While this behaviour has been interpreted as a way of 'accumulating mutations' (Segré *et al.*, 2000a), the (inevitably) limited lifetime of a composome may also very much discourage its evolution. Also, it is difficult to grasp how ever more new mutations can actually arise and are accumulated, i.e. how complexity is further increased, considering that the system just switches from one stable state to another. A certain composome may be selected for, however evolution seems to stop there: the number of end-points is only as big as the possible number of attractors in the system (Szathmáry, 2006), which is much smaller than the imagined number of vesicles in the prebiotic environment. E.g. Markovitch and Lancet, 2012 show that with parameters set to ordinary values, the mean number of composomes lies between 1 and 4 (depending on the pre-fission assembly size (N_{max}): the smaller the vesicle, the higher the composome diversity), with maxima not exceeding 9 (Figure 1C); by no means a large possible diversity for natural selection to act upon. The 'evolutionary tree' that is shown by Segré *et al.*, 2000a thus possibly depicts selection, but not evolution in an open-ended manner (for a short discussion on the nature of inheritance and open-ended evolution, see Fernando *et al.*, 2011).

Concluding: it is far from evident that (1) composomes did arise on early Earth, and (2) they evolved to something far more complex.

Non-evolvability of GARD

Eörs Szathmáry and coworkers addressed the very first evolutionary steps of GARD ensembles and conclude that selection cannot maintain the fittest vesicles (Vasas *et al.*, 2010), which would mean that even evolution in a closed manner is impossible in the lipid world scenario (and real open-ended evolution is even further off). In the words of Szathmáry and coworkers: the system lacks evolvability.

To arrive at their conclusion, an environment with only 10 different kind of molecules was constructed (N_G=10; GARD assemblies are thus characterised as a 10-long vector in composition space). Vesicles with N_{min}=3 were allowed to grow and split at $2N_{min}$. The small system size allowed for an exact solution to the replication-mutation equilibrium distribution of all possible assemblies, by constructing an Eigen equation for the GARD model (the quasispecies model was extended to compositional assemblies; see Vasas *et al.*, 2010). For any 10-long vector with N_{min}=3, every possible progeny assembly of the same size was calculated. A fitness matrix was constructed, where off-diagonal elements indicate net mutant production, and diagonal elements indicate net exact reproduction. Subsequently, the frequency distribution of the stationary population of compositional ensembles, i.e. the background rank order distribution without imposing selection, was drawn.

Next, selection pressures were introduced. A target was defined and the growth rate of an

assembly was increased or decreased based on (dis)similarity to the target. Two different targets were considered: one at low replication-mutation equilibrium in the background distribution and one near the top (ranked 5th).

While the frequency of target assemblies changed slightly relative to the background frequencies, the rank order hardly changed. In case of the low-ranking target, the target assembly still stayed in the tail of the distribution after selection; in case of the top-ranking target, the first 24 assemblies in the background distribution remained exactly the same. Szathmáry and coworkers therefore conclude that 'imposing Darwinian selection to the GARD model has, at most, negligible effects on the background distribution defined by the asymptotic steady-state solution already built-in' (Vasas *et al.*, 2010, p. 1473). Subsequently, the same was demonstrated for larger assemblies (N_{min}=40; N_G=100), albeit in a different (statistical) manner: no matter what composition was chosen as a target (after performing a principal component analysis of the covariance matrix of molecular concentrations of the late-time stationary distribution (higher eigenvalues correspond to compositions that recur in the time-dependent dynamics), the first 10 principal components, explaining 99,7% of the total variance, were used as a target in a population of 1500 assemblies), the time-dependent trajectory of a population hardly changed as compared to background dynamics. Some assemblies are thus selected for in the model, however not by the imposed selection (replication of composomes is too inaccurate to maintain the fittest vesicle), but by the underlying dynamics of the β matrix (the reason being 'hidden compartmentalisation' in the β matrix, not discussed herein but the reader is referred to Vasas *et al.*, 2010). *Ergo*: compositional ensembles cannot evolve.

Criticism to the non-evolvability of GARD

Lancet and coworkers responded to the claim that GARD assemblies cannot evolve (Markovitch and Lancet, 2012). Their main criticism to the reasoning of Vasas *et al.*, 2010 appears to be a valid one, namely that not quasi-stationary-states in composition space, i.e. composomes, were used as target, but instead random compositions. Indeed a rank order was constructed out of all the possible compositions. As was described above, only composomes are supposed to be the ancestors of life and evolvable entities in the lipid world scenario, not any random composition of lipids. A certain level of selection is obviously already implemented by the β matrix, in the sense that composomes appear more frequently than random compositions: selection towards any composition that is not a composome is inherently impossible. However, this is not the kind of selection that is envisioned for evolution in the lipid world scenario; selection among quasi-stationary-states is. While the targets used by Vasas *et al.*, 2010 *may* be composomes, this has not been shown. Non-quasi-stationary-state compositions might be frequent because they are produced by a dominant composome, and one compotype may comprise multiple composition. The least that can be said is that the low ranking target probably is not a composome, considering the small assembly and N_G size. On the other hand, it is difficult to believe that in the population model with the large assembly size, no targets were composomes, given that 99,7% of the total variance was explained by the target compositions; although theoretically, they could e.g. all be part of one compotype. In any case, it has not been addressed whether or not the target compositions were composomes, and the conclusions of Szathmáry and coworkers cannot conclusively be regarded as evidence for the non-evolvability of GARD.

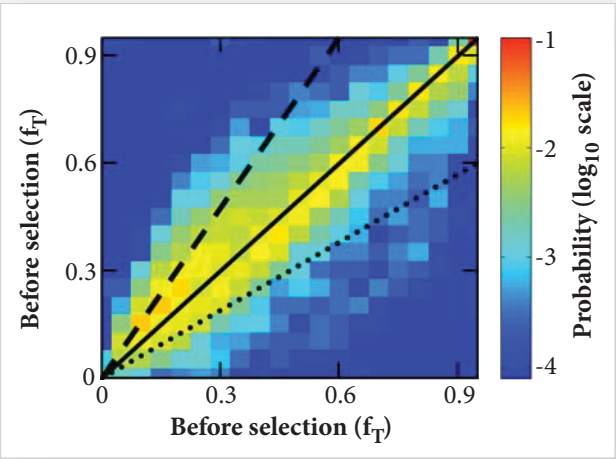
Lancet and coworkers claim that if quasi-stationary-states are used as selection target, compositional ensembles do react significantly to selection, especially when a high proportion of mutual catalysis (as opposed to autocatalysis) is present in the ensembles (Markovitch and Lancet, 2012). They proceeded as follows.

In a single vesicle GARD simulation, composomes were identified and underwent *k*-means clustering. The centre of mass of a cluster was defined as a compotype, resembling the quasi-stationary-states that should be selected for in the lipid world scenario. The most frequent compotype was chosen as a target, and a fitness gain was implemented by the direct enhancement of the β values depending on similarity to the target after every fission event. 10,000 simulations were run in total, first without and then with selection. Indeed, if the frequency of the target compotype before selection is plotted against its frequency after selection, an overall skew towards positive selection is seen (Figure 2A). Interestingly, a significant amount of negative selection is also present, meaning that selection towards

the most occurring compotype leads to a decrease in frequency of that compotype. Also, in 1000 simulations of a population of 1000 assemblies under constant population conditions (thus, as opposed to the previous analysis, more than one GARD assembly and hence competition was considered), the target compotype becomes more frequent under selection pressure in 50% of the cases, with still negative selection in 15% of the cases, the remainder being no selection at all. If fitness advantages are calculated at every time point instead of merely when the vesicle splits, overall selection approaches even higher positive values (70%).

The parameters that influence this 'evolvability' (or perhaps better: selectability) are also discussed: especially the extent of mutual catalysis and N_{max} strongly affect the system's ability to undergo the proposed kind of selection, both positive and negative. In other words, very special conditions are needed not only for the existence of composomes, but also for the possibility of their selection.

It is tempting to interpret the above-stated as evidence for the possibility of evolution in the lipid world. However, in particular the fact that only the *already most occurring* compotype was chosen as a target, makes such a conclusion as yet unsupported: only if it is shown that any compotype, also a low ranking one, can be selected for, will the hypothesis stand strong. The existence of 'choice', in the sense that multiple compositions can be sampled by natural selection, is a hallmark of evolution. A pronounced change in rank order distribution due to selection as was envisioned by Vasas *et al.*, 2010, albeit of compotypes instead of compositions, would be the first convincing piece of evidence for the possibility of evolution in the lipid world scenario. None such evidence is given. The presence of selection in a negative direction would even suggest the opposite: in some cases, even the most frequently occurring compotype cannot be selected for. Unfortunately, these dynamics were not further investigated.



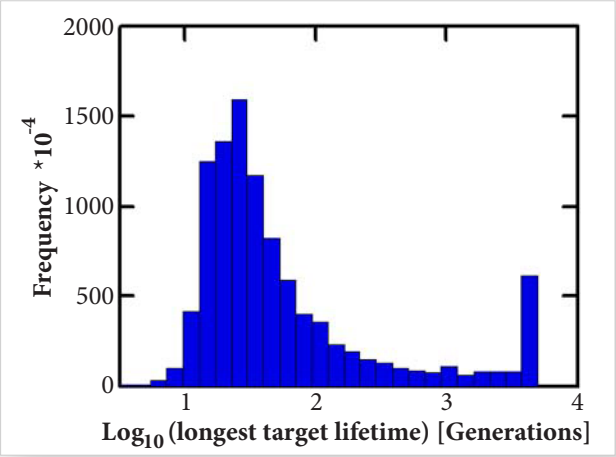


Figure 2. (A) The correlation between the frequencies of a target compotype before and after selection shows an overall skew towards positive selection (A). Colour represents probability out of the entire dataset of 10,000 simulations. Positive selection is depicted above the solid line, negative selection below. Dashed and dotted lines represent selection excesses of 1.5 and 0.5, respectively. The distribution of the longest per simulation target compotype lifetime is highly skewed towards shorter lifetimes (B). Note the log_{10} scale of the x-axis. The frequency peak in the rightmost bin can only partly be explained by the distorting nature of the log_{10} scale of the x-axis. The bin comes close to or encompasses the total number of generations in the simulation (5000). It is not addressed by Markovitch and Lancet, 2012. However, it seems to be driven by simulations with an overall low compositional diversity, i.e. in which a single compotype dominates, as suggested by the bimodal distribution of the similarity parameter H_0 (Markovitch and Lancet, 2012, Figure 7B), and its dependency on a low number of compotypes for higher values (Markovitch and Lancet, 2012, Figure 16B). Figures from Markovitch and Lancet, 2012.

Also note that if, in a single assembly under selection, a certain compotype becomes more frequent, still its composition cannot be maintained throughout the entire simulation, due to the stochastic splitting of the vesicle. It is therefore highly doubtful whether the presence of a target compotype in population models is always due to inheritance from earlier generations: the compotypes may result from ever new compositions instead of a common ancestor (in the sense that if the composition is lost, selection can start all over again). In fact, a first real indication of the lifetime of compotypes is given. The most frequent whole-simulation average compositional lifetime is 3 generations, with some simulations exceeding 10 generations (the compositional lifetime is an interpretation of the parameter τ, see Equation 7 in Markovitch and Lancet, 2012). It is stressed that the compositional lifetime is not the same as the composomal lifetime, and that composomes do live longer than random compositions:

> 'In fact, the most probable target compotype lifetime (taking for simplicity the maximal time from each simulation) is 30, and the average is 434 generations.' (Markovitch and Lancet, 2012, p. 252)

It is unclear what makes the use of the maximal lifetime simpler than e.g. the more useful average lifetime; the statistic merely fogs the analysis (a possible reasoning could be that only one long lifetime is needed; a more valid argument however would be that one does not need one long lifetime, but instead many possibilities for evolution to occur). Also, the average of 434 generations is highly biased by few long lifetimes, and the distribution is actually skewed towards shorter lifetimes (Figure 2B). Still, if even the longest living composomes persist for only 30 generations or so (or at least for an order of magnitude of 10^1–10^2 generations), it is hard to envision how composomes can provide a stable base for evolution: every so many generations, evolution may need to start all over again. The scenario seems to depend on a lucky accident moreover, considering that only a universe with one of the few sufficient β matrices would have seen compositions of any interest, namely ones that live passed a few generations.

Concluding: while it has not been convincingly demonstrated that the GARD model lacks evolvability as was claimed by Szathmáry and coworkers, it has not been proven that it can be subjected to selection either. Serious doubts regarding evolvability remain in existence.

Conclusion: did life start as an evolvable lipid vesicle?

Despite more than a decade of research, the lipid world hypothesis, arguably the most elaborate metabolism-first theory that proclaims the Darwinian selection of network replicators, still hangs on the very first steps. Besides the absence of evidence from wet experiments or even a direct link to real chemistry, a proof of concept has until today not been given. Indeed, an answer to the question how far the lipid world scenario has come in an explanation for the origin of life must be: not that far. What has been shown is that composomes can arise if the conditions are right, and that they can exist for some generations. In other words: there may have been (with a very strong emphasis on *may*) lipid vesicles on early Earth whose composition stayed roughly the same for some time. However, evolution of composomes, the distinctive characteristic of the scenario, poses a serious problem. By no means has it been shown that a system of ensemble replicators could eventually have given rise to a system in which information was stored in replicating polymers. Although proponents might feel different, the rudimentary hereditary dynamics of composomes do not inevitably guarantee its evolution. In fact, most indications described above suggest that lipid ensembles simply cannot increase in complexity by the process of evolution. The most urgent open questions for the lipid world scenario that should be answered first are hence focused on evolution:

1. Can vesicles be selected for (taken into account all the questions posed above, e.g. on the limited lifetime of composomes)? How does the composition of a vesicle relate to its function and consequently:
2. How does the system become evolvable in an open-ended manner? Obviously, a way of introducing and accumulating novel mutations must be implemented. Modelling has hardly been concerned with the question (a case can be made for answering (1) first before addressing

the question of open-ended evolution: if selection of composomes is impossible, then the question of open-endedness is no longer relevant, along with the entire scenario). Interesting extensions to the GARD model might provide a clue (Polymer-GARD (Shenhav *et al.*, 2005) and Exchange Polymer-GARD (Shenhav *et al.*, 2007); extending GARD beyond the monomer world would obviously open up possibilities), yet are for now discontinued. One could argue that no open-endedness is needed, and that merely selection among few (up to now it appears less than 10!) composomes provides enough base for the origin of life. However, the notion means nothing if one does not specify how the system becomes open-ended thereafter, e.g. how the RNA world arose from the lipid world. In other words: 'what was the function of a lipid vesicle?', is a most important question that should be answered in the near future. How did a vesicle its composition aid in the emergence of life? If it only provided a stable environment for molecules to interact, then its possible evolution is not necessary for an explanation to the origin of life (and not a lot of open-ended evolution is needed indeed). Eventually, the scenario should be able to account for the leap to information storage in polymers, however questions of evolution and function are addressed too little in the scenario. They should be central for research to come. The recent development of Universe-GARD (Markovitch *et al.*, 2012) is a step in the right direction, at least in the sense that the problem of open-ended evolution is acknowledged. However, the proposed solution appears like cheating. The surrounding is just elaborated as compared to the imagined vesicle; still, if the entire universe is concerned, possible directions for evolution remain limited due to attractor-based inheritance. While the number of attractors may be enlarged, the question of the path to open-endedness, related again to function, lingers.

3. Atop these questions, the boundary conditions that are needed for the scenario should be investigated. As was shown, very special early Earth conditions are needed for the existence of composomes and possibly even greater restrictions to allow for their selection (if at all possible). Eventually, even if the scenario turns out to be possible, the likelihood that life on Earth started with a lipid world should be addressed.

The above-stated questions arise in the lipid world scenario, but are topical for any scenario that proclaims that the first Darwinian entities were metabolic networks. How to get from a metabolic network to something far more complex represents Achilles' heel of these metabolism-first scenarios. Earlier theories have been abandoned mostly because it was unclear how to continue after the self-organisation step. The problems of evolvability arise because in ensemble replicators heredity is inaccurate and, *even if* accurate enough, attractor-based. Any theory should deal with these problems, one way or the other. In the next section, one possible albeit highly theoretical, i.e. far off from real chemistry, way of accumulating mutations in ensemble replicators is discussed. It is the only demonstration to date of the possibility of evolution in a metabolism-first scenario.

3.2 Accumulating mutations in replicating networks

Citing their previous work on the non-evolvability of composomes, Szathmáry and coworkers, among which Stuart Kauffman this time, conclude that autocatalytic sets as envisioned in contemporary metabolism-first scenarios, no matter what the chemistry, cannot be units of evolution (Vasas *et al.*, 2012). They aim to show the general requirements necessary for reaction networks to be units of evolution, using the old polymer model of Doyne Farmer (Farmer *et al.*, 1986) as an example. A protein network is readily imagined, but its concept can in principal be applied to any polymer network, even to RNA networks; one is then dealing with RNA species that are not replicators themselves. An important requisite is compartmentalisation of the chemical networks, which allows for selection against disadvantageous modifications. Szathmáry and coworkers provide the first and only demonstration of how chemical networks could accumulate mutations.

First, the anatomy of an autocatalytic set is analysed. An autocatalytic set can be divided into one or more strongly connected cores, and their peripheries. The provision of a core species suffices to produce (i.e. catalyse the production of, in the Farmer model by cleavage or ligation reactions) all core species together with the periphery species, while the periphery species cannot ignite a core or periphery themselves; their existence in the set depends on catalysis by the core species only. Cores can be connected: a species in one core can be the food molecule for another core, or cores can share a periphery (Figure 3). The reader is referred to Vasas *et al.*, 2012, Figure 1, for a vivid understanding of these concepts.

It is subsequently shown that compartmentalised polymer networks that contain only one autocatalytic core, the networks that arise in the original Farmer model, always have one attractor, and selection is thus impossible. The dynamics are deemed analogous to that of the GARD model, wherein composomes inevitably form only one core, and eventually only one attractor exists. Based on this, autocatalytic networks with only one viable core, basically all networks thus far proposed in scientific literature, are discarded as units of evolution. While, as described above, the conclusions of Szathmáry and coworkers regarding the GARD model can be disputed, one is inclined to follow their reasoning now, at least to the extent that evolution in GARD has not been demonstrated, and that the model in its current form either way would not get one very far, i.e. past one attractor, whether selected for or not.

Despite the non-evolvability of single-core networks, it is shown that if rare non-food species are occasionally added to the system (the species emerge from uncatalysed reactions out of existing species), selection is sometimes possible. Whenever the addition of novel species results in the ignition of a new viable loop (i.e. a novel core), multiple attractors always exist. In these cases, selection among attractors is possible. The new core leads to a new attractor for the network (basically, adding a novel core leads to a distinct stable composition, namely the two combined attractors), and represents a selective advantage due to a higher growth rate of the entire system; after all, the core is autocatalytic. The result is that the novel network represents the most frequently encountered network type. However, a mere 1% of artificial selective advantage for the network without the novel core would result in a significant reduction of networks with the viable core in the population. Hence, two exponentially growing entities with different growth rates are there to choose from by natural selection.

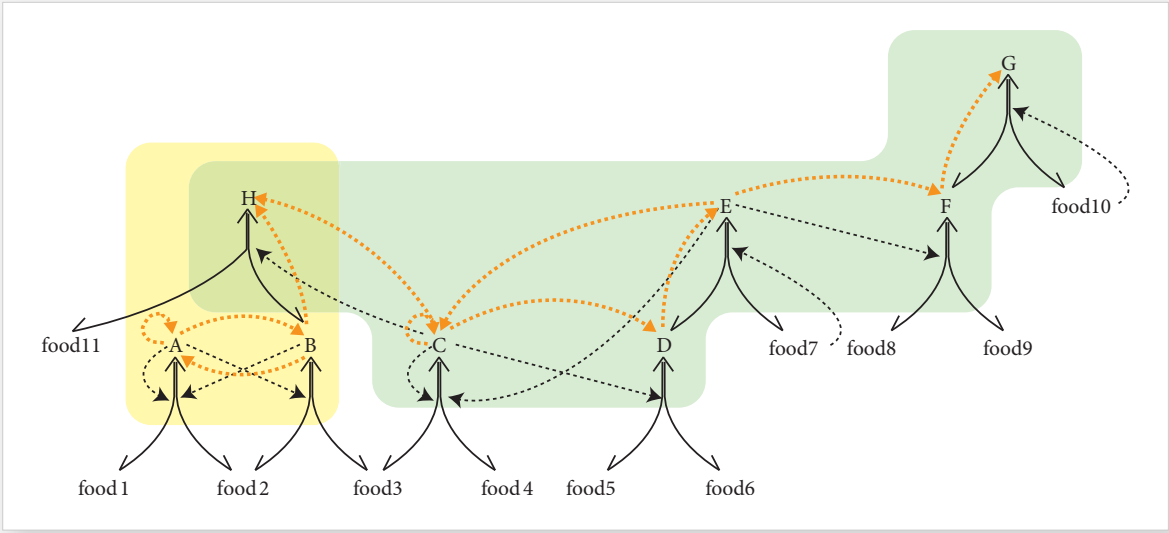


Figure 3. Multiple cores in autocatalytic sets allow for selection of chemical networks. Solid lines are reactions, dotted lines are catalytic activities. Orange dotted lines depict autocatalytic loops. Food molecules are assumed to be present at all time. A/B and C/D/E are two independent cores (if one of the molecules arises spontaneously, the core and its periphery emerge), with F/G/H as periphery. The cores are connected via the shared periphery H. Three possible attractors are present: either one of the independent cores, or the combination thereof. Figure from Vasas *et al.*, 2012.

Rare species could again give rise to novel viable cores, that can have both positive and negative effects on the growth rate of the entity, or any other property that is related to fitness. Note that also the species in the periphery may contribute fitness-related properties. Due to possible asymmetric splitting of compartments (just as in the GARD model), cores and their peripheries can again be lost.

The path to template-dependent replication

In the proposed networks, a viable core represents one bit of heritable information: the loop is either there or not. Therefore, the amount of selectable attractors in the system appears small, and the model might not be able to account for the much looked for open-ended evolution. However, to relate to the work of Lancet and coworkers, the number of possible single core attractors in the polymer system, i.e. the number of compositions that can make up a novel core, may be way larger than the number of possible attractors in the lipid world (whether they can be selected or not) because composition space is ultimately much more restricted in the monomer world of GARD. While technically an attractor only comes into existence if a non-food molecule spontaneously emerges, the potential number of attractors that might is thus high. Obviously, if cores can be accumulated, the possible number of attractors in the system is enlarged, with some inherently having a higher complexity than others: the ones that contain multiple cores. Moreover:

> 'Whenever novel spontaneous reactions occur, the number of possible uncatalyzed reactions also increases [note that this is the source of novel species], opening up new possibilities for discovering viable cores (genotypes) and their corresponding peripheries (phenotypes).' (Vasas *et al.*, 2012, p. 10)

The number of possible attractors becomes thus ever larger because the system itself generates novel building blocks. Therefore, the system may be characterised by expansions into reaction space, providing at least some way of step-wise innovation. Ultimately, the 'open-endedness' of the model, much more open-ended than the lipid world but less than the RNA world, depends on the nature of combinatorial polymer chemistry. Monomer-based models like GARD are simply bound to the number of possible monomers in the environment, while the polymer model proposed herein is bound to the possible number of polymers, that represents only a function of the number of monomers in the environment. The polymer model ensures an almost unlimited number of theoretical possible species and thus reactions from one food set. It is interesting to relate this to the solution to 'open-endedness' of Markovitch *et al.*, 2012 in the GARD model, wherein novel monomers must be added from the environment. The combinatorial chemistry together with the property that one entity can contain multiple cores gives rise to these rudimentary evolutionary dynamics in the model.

Evolution of this kind could account for an initial increase in complexity, and perhaps eventually for a transition to a template-dependent replicator world. It can be imagined that RNA could have first taken over some functions, perhaps redirecting its role from early parasite to dependent molecule, before assuming its role as information carrier.

There is however much to be done before one is inclined to believe that networks as proposed by Vasas *et al.*, 2012 seeded modern life. The crucial problem is perhaps that the step to realistic chemistry seems to be a leap at least. The existence of the imagined autocatalytic polymer networks has not been demonstrated; Vasas *et al.*, 2012 can only refer to the conceptually different peptide networks of Ashkenasy *et al.*, 2004. As said, evolutionary dynamics are very much dependent on the combinatorial chemistry of polymers, excluding most probably non-polymer systems as a viable solution: even though the concept of accumulating viable cores may be applied to monomer networks, dynamics will be different and much more limited in non-combinatorial chemistry. With it arise the same questions about the probability of a polymer world that afflict replicator-first scenarios. Moreover, it seems a problem that in order to get to higher complexity, ever more cores must be added to a network that encounters difficulties in transferring a large amount of information over generations. Just as in the GARD model, the higher the network diversity of an entity, the less likely it is that all of its contained information will be transferred to progeny. It is a legitimate question whether the proposed model will get one far enough to overcome the problem of inaccurate information transfer.

Unfortunately, the question was not addressed by Vasas *et al.*, 2012. In addition, more common problems such as the question of compartmentalisation (after all, a crucial feature of the model is that the networks are compartmentalised) related to protocell problems in general such as that of molecule transport across early membranes (but see Mansy *et al.*, 2009), and stability of the system in real life will have to be addressed.

3.3 The standing of evolvable metabolic networks

The standing of the possibility of Darwinian selection in metabolism-first scenarios for the origin of life can be regarded as problematic. The most elaborate theory, the lipid world scenario, appears to be somewhat realistic at least as far as the involved chemistry is concerned, but has not yet given a proof of concept, and it is doubtful whether it ever will. Whereas the only theory that has given at least some proof of concept is very far off from real chemistry, and is accompanied by many open questions, of which some essential ones are shared with the replicator-first scenario. One can only agree with Szathmáry and coworkers, who note that:

> 'At the time of writing then, we are still a long way from knowing whether autocatalytic sets offer a plausible model for the emergence of evolvability.' (Vasas *et al.*, 2012, p. 2)

To refer to the replicator-first scenario: finding a viable path to the prebiotic synthesis of the first replicator molecule might be difficult, but as for now, no scenario provides any viable easier to emerge evolvable entity. The questions and remarks that are provided in this paper may provide guidance for either the final rejection of the scenario or a possible way out of the encountered problems.

3.4 Life as a necessity

Eric Smith and coworkers (notably Harold Morowitz, who started the line of reasoning years before Smith appeared on stage) propose a theory for the origin of life that is a true metabolism-first theory in the sense that it proclaims that the first important step for the emergence of life was the onset of metabolism (Smith and Morowitz, 2004; Morowitz and Smith, 2007; Morowitz *et al.*, 2008; Morowitz *et al.*, 2010; Smith and Morowitz, 2010; Braakman and Smith, 2012b; also vividly expressed in the semi-scientific journal American Scientist (Morowitz and Smith, 2009); see also earlier writings of Morowitz (e.g. Morowitz, 1999)). The theory states that part of metabolism in contemporary life is the most ancient attribute of life, thus differing from the priorly discussed theories in which the analogy with metabolism went only as far as an autocatalytic set of non-replicating molecules. It is a preparatory metabolism theory in the sense that the first metabolic network is not imagined to evolve.

What makes the theory interesting at first sight is that it provides more than an explanation for the origin of life. It provides an intriguing view on life in general, and on the roles of chance and necessity in it. Instead of answering the question: 'how did life arise?', the theory aims to answer the question: 'why is there life instead of non-life?', thereby implicitly addressing the first question. Metabolism plays a fundamental role in the proposed hypothesis.

Not only was metabolism the first important step in the emergence of life, it is also the single most important attribute of life, the theory states (as opposed to e.g. the notion of individuality (Morowitz *et al.*, 2008)). Instead of metabolism being 'just another' property of life, life is claimed to be an inevitable consequence of Earth's geosphere via core metabolism. As such, life its emergence was a necessity, instead of a lucky event. It could not have gone otherwise. The idea that the origin of life is a necessity is not new, however Smith and coworkers provide a testable hypothesis.

The theory is arousing, especially because it bridges the gap between life (the biosphere) and

non-life (the geosphere) via the concept of energy flow: energy differences generated by the geosphere act to organise matter into a biosphere. It is appealing because it provides a simple and elegant solution to the origin of life enigma: the origin of life is reduced almost to a law of physics. Also, it puts to the test the kind of explanations that are generally searched for, and the questions that are asked, for a solution to the origin of life enigma.

Being arousing and appealing alone does not make any idea true, and it is shown that the theory rests on assumptions that have not yet been proven, and may ultimately be a misinterpretation of facts. However, it is argued that the theory provides an interesting solution to the restricted biochemistry of today, that is worthwhile future investigation.

Life and energy

Eric Smith's ideas are perhaps best summarised in the introduction of Morowitz and Smith, 2007:

> 'Life is universally understood to require a source of free energy and mechanisms with which to harness it. Remarkably, the converse may also be true: the continuous generation of sources of free energy by abiotic processes may have forced life into existence as a means to alleviate the buildup of free energy stresses. (…) It would show that a part of the order we recognize as living is thermodynamic order inherent in the geosphere, and that some aspects of Darwinian selection are expressions of the likely simpler statistical mechanics of physical and chemical self-organization.' (Morowitz and Smith, 2007, p. 51)

An analogy is subsequently drawn with lightning bolts and hurricanes: physical processes that channel matter and energy far from equilibrium between different energy reservoirs at much higher rates than through near-equilibrium states. Smith and coworkers believe that life is the non-equilibrium channel state that reduces energy differences generated by the geosphere, and as such is just as inevitable to happen as lightning or hurricanes.

The free energy stress needed for the initial release of the channel state is thought to be generated by geochemical processes. Carbon dioxide (an oxidant) and molecular hydrogen (a reductant) are continuously generated in geothermal environments like hydrothermal vent systems (whose discovery in the '70s (Corliss *et al.*, 1979) provided a fertile breeding ground for origin of life theories; see also Martin *et al.*, 2008, who unfortunately only briefly touch the origin of life theory of Smith and coworkers, but do provide a comprehensive overview of the chemistry of hydrothermal vent systems). The lowest energy state of the couple is represented by methane and water: in this state all electrons are transferred from hydrogen to carbon dioxide, leaving the system devoid of electrons that can be transferred and thus provide energy. However, in the absence of life, the reduction of carbon dioxide does not take place at any interesting rate. The molecules pile up in the environment, hence generating a free energy reservoir. It is suggested that the origin of life 'solved' this free energy 'problem' of the geosphere (and later on also other free energy stresses, among which that generated by sunlight), by transferring the energised electrons to biomass (Morowitz and Smith, 2007).

Living organisms can indeed reduce inorganic carbon with remarkable efficiency, but they do so with the aid of a complex set of molecules. However, Smith and coworkers believe that the reverse tricarboxylic acid cycle, the same metabolic carbon reducing cycle that is also found in modern reductive chemo-autotrophs, arose spontaneously in reducing high-energy (i.e. non-equilibrium) environments. In order to understand the argument, one must understand the structure of contemporary metabolism.

The anabolic core of all life

The reaction networks of all contemporary species can be mapped into a single chart, that is based on less than 500 small organic molecules, representing the universality of intermediary metabolism within the great diversity of life (Morowitz *et al.*, 2000). A distinction within core metabolism is made between anabolic and catabolic processes (Braakman and Smith, 2012b): anabolism builds organic compounds from smaller units (in a reductive process: consuming electrons to create molecular bonds) and catabolism breaks down organic compounds, thereby producing energy in the form of energised

electrons and carbon dioxide. An anabolic organism can exist completely on its own, given that electron donors are present in the environment (of which contemporary chemo-autotrophs are superb evidence), whereas catabolism is associated with heterothrophy. However, any heterotrophic system also has an anabolic core, that is powered by catabolism. Note that photosynthesising organisms, even though autotrophic, have evolved ways to store and catabolically break down organic molecules. Catabolic pathways may differ between ecosystems (and may be absent altogether in a chemo-autotrophic system), however an anabolic core is present in every system, and is thus the one truly universal feature of core metabolism (Smith and Morowitz, 2004; Figure 4A).

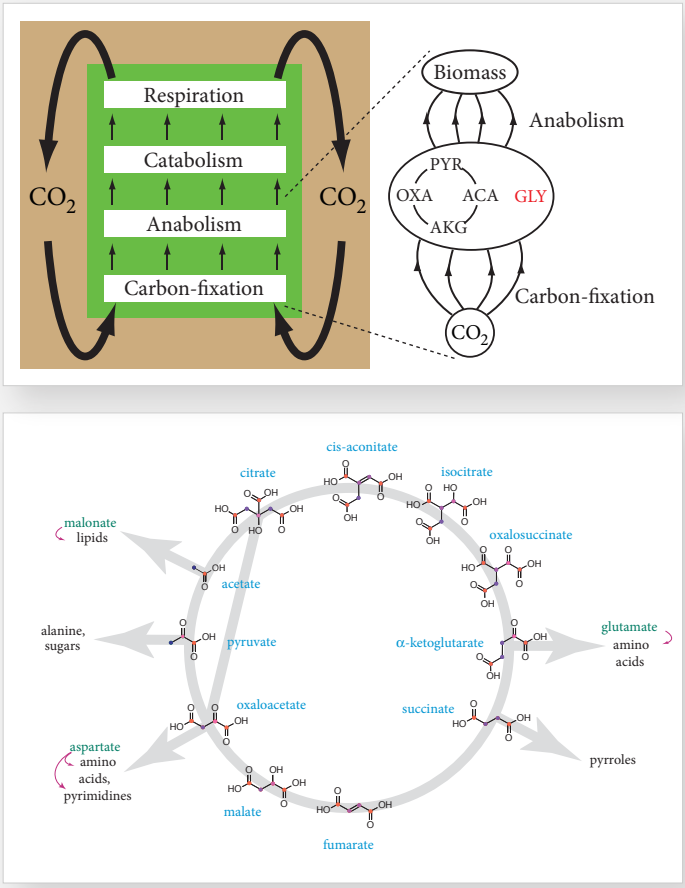


Figure 4. Carbon cylcing in the biosphere is ultimately based on carbon-fixation and anabolism (A, left). The interface between carbon-fixation and anabolism is universal and consists of a small number of organic molecules (A, right): the intermediates in the TCA cycle (B). Every major anabolic pathway arises from one of the molecules. Nowadays, the molecules are best known for the catabolic TCA cycle (C, left), but in reductive chemo-autotrophs, the cycle can run in the reverse anabolic direction. Eric Smith and coworkers believe that life started with a primordial rTCA cycle, in which carbon dioxide was reduced to more complex molecules (C, right). Figures from Braakman and Smith, 2012b (A,B), Morowitz and Smith, 2009 (C).

Every anabolic pathway in core metabolism arises from one of 11 carboxylic acids of the tricarboxylic acid (TCA) cycle. To appreciate the observation to its full extent: every single organic molecule in living organisms, all of the biomass on Earth, originated in one of these molecules (Figure 4B). The molecules are best known from the TCA cycle, also called Krebs or citric acid cycle, in which organic molecules are oxidised to carbon dioxide in photo-autotrophs and oxidising heterotrophs (Figure 4C, left). The cycle as such is catabolic, extracting energy from the molecule PEP, that is ultimately derived from complex photosynthetic pathways. However, in a reducing environment, the cycle can run in the reverse direction, thereby producing organic molecules from carbon dioxide, extracting energy from electrons in the environment; the way of making a living for modern reductive chemo-autotrophs (Srinivasan and Morowitz, 2009a). Perhaps not so incidentally, chemoautotrophs encompass the oldest clades of life.

Smith and coworkers argue that life started with the molecules of the TCA cycle. Considering that Earth's atmosphere was not oxidising until at least ~2 billion years ago, as evidenced by banded iron formations, it appears very unlikely that the cycle could have operated in the oxidative mode early in the history of life (Smith and Morowitz, 2004). Complex life must have managed to survive well before the TCA cycle was around. Also, photosynthesis appears to be too complex to start life with. Instead, it is stated that life started in the reducing high-energy environments described above (that were probably very abundant early in Earth its history), and that its very first emergence was that of the anabolic rTCA cycle, albeit in a preenzymatic primordial form, having the obvious benefit that in such a scenario the capture of energy and the synthesis of biomass are directly related (Figure 4C, right).

The origin of the rTCA cycle and the rise to complexity

It is shown that in a strongly reducing environment, all molecules of the rTCA form a relaxation pathway for the surplus of free energy (Smith and Morowitz, 2004). Smaller molecules along the pathway from carbon dioxide to methane are unstable against collapse to cycle intermediates. Hence, on the reduction sequence from carbon dioxide to methane, smaller molecules need not be more stable than large molecules. No molecules appear to be as energy efficient as the molecules of the rTCA cycle. Concluding: even though rTCA molecules are moderately complex, this does not mean that their demolition in side-reactions is favoured. It is hypothesised that the rTCA cycle is the favourable (in a thermodynamic sense) relaxation pathway for the free energy reservoir generated in geothermal environments.

Moreover, the rTCA cycle can duplicate its intermediate molecules, and as such has autocatalytic properties (Braakman and Smith, 2012b; the nature of this 'autocatalysis' is discussed further on). If the simplest compounds arose spontaneously and the cycle was indeed thermodynamically favoured, its network topology ensured self-amplification. All of energy flow and all of inorganic carbon in the system would have gone through the cycle, resulting in persistent order.

Even though the chemistry may be very interesting by itself, one is probably not inclined to call the cycle life, nor admit that the origin of life enigma is solved by its emergence. From the three questions posed in the beginning of this paper, only the first question might have been answered, however a solution to the origin of life should address all three. It is thus necessary to pose the question how life continued after the emergence of the cycle, even though the focus in the scenario has been placed more on the rTCA cycle than on subsequent steps.

From the intermediate molecules of the persistent rTCA cycle, more complex molecules may have formed (as will be discussed later, small catalysts may be abundant in geothermal environments). Note in this respect also that the end-product of the rTCA cycle is acetate, not methane, thus leaving room for further carbon reducing steps like the (non-autocatalytic) formation of fatty acids. The cycle would have provided the starting molecules for novel pathways, in a very stable fashion. Importantly, these pathways are thought to be in general biological useful, i.e. metabolic rate enhancing, instead of parasitic, basically because any pathway that enhances the output of the metabolic core is favoured over one that does not (see Smith and Morowitz, 2004). Such a scenario is only viable however, if the pathway is either thermodynamically favoured or there is an (in)direct coupling between the network and the catalyst of the novel pathway (it is not always clear which solution Smith and coworkers have in mind). In the early steps of life, cofactors are imagined to play a critical role (as they still do today;

not further discussed herein, but the reader is referred to Morowitz *et al.*, 2006; Braakman and Smith, 2012b). The important notion is that the rTCA cycle provides a steady supply of starting molecules for anabolic pathways.

One possible novel pathway is provided by Copley *et al.*, 2005 and Copley *et al.*, 2007, who propose a scenario for the origin of the RNA world. The scenario is fairly chemical, and only the concept is discussed herein. An important assumption is that the nature of hydrothermal vent environments, namely environments with steep temperature gradients and many cavities resulting in distinct micro-environments, allows for the formation of fragile molecules at some places, as well as for compartmentalisation that prevents dilution. It is hypothesised that primordial hydrothermal vent systems contained, besides a functioning rTCA cycle, a myriad of organic molecules that could have exerted at least some catalytic power (perhaps aided by mineral surfaces, see further on), along with simple molecules such as ammonia.

An autocatalytic loop between the synthesis of nucleotides, derived from amino acid precursors, and nucleotide catalysed amino acid synthesis from α-keto acids that find their origin in the rTCA cycle (Copley *et al.*, 2005), is proposed as the novel invention that persisted because it enhanced the flux of matter through a self-amplifying pathway, and might have been favoured because metabolites participated in substrate-assisted catalysis of the rTCA cycle, or enhanced metabolic cycling because they attached to small catalytic molecules (only briefly mentioned in Copley *et al.*, 2007; note in this regard that it is doubtful whether the cycle as a whole spins faster if merely one of the steps is catalysed, and catalysis must thus have been not very specific at this stage). A series of steps to template-dependent replication is proposed (Copley *et al.*, 2007) in which the fitness of oligomers depends on the ability to support existing pathways rather than self-replication (Smith and Morowitz, 2010).

Interestingly, the addition of a novel viable loop to an existing pathway shows some resemblance to the accumulation of mutations in the polymer model discussed earlier. Layers of complexity may be added to the first persistent cycle, in a form of chemical evolution. Self-replication is a property of the network instead of a property of single molecules. The fitness criterium would ultimately be the rate of anabolic cycling. Smith and coworkers have dubbed the term 'selfish metabolism' for the early steps of life (Morowitz *et al.*, 2008), however it is unclear whether evolution of networks is imagined, or things fall into place like a lightning bolt, i.e. most of metabolic pathways and the combination thereof were the product of thermodynamics and chemistry alone. Terms like 'fitness' may be infelicitous.

The key question in this regard, and indeed for the entire scenario, is how much order is due to geochemical processes. In other words: how much complexity can be explained by thermodynamics? In the world imagined by Smith and coworkers, it is not quite clear to what extent necessity lingers:

> 'It is likely that cells came into existence as the most productive platform for metabolism, after which the characteristics of individuality and heritable variation so central to Darwinian selection emerged as a by-product of cellular form.' (Morowitz and Smith, 2007, p. 56)

Which suggests that Darwinian selection is imagined to have started only after cellular compartmentalisation (note that the imagined cells are not the spontaneous forming simple lipid droplets, but cells that arose as a result of metabolism), and that before it, only thermodynamic ordering mechanisms were at work. In other words: thermodynamics needs to account at least for the pathway to lipid synthesis (or in fact: for the pathways that lead to a functioning (metabolic-rate enhancing) cell). As a side note: as was discussed in the first part of the present paper, Darwinian selection of metabolic networks might be less evident than assumed by Smith and coworkers.

Did life start as the rTCA cycle?

As yet, evidence is lacking. The strongest argument against an inevitable origin of life is of course the absence of spontaneous emergences nowadays, if not in the contemporary geosphere (one could argue that the existence of the omnipresent biosphere prevents new emergences), then at least in the test tubes of laboratories.

The first thing to notice is that the rTCA cycle is not truly autocatalytic. It may be self-reproducing in the sense that its molecules are duplicated if the cycle progresses, however it only has this property embedded in the complex network of contemporary biochemistry (the cycle thus differs from priorly discussed autocatalytic networks, in which every reaction step in the cycle is catalysed by a molecule within the cycle itself). It was shown that the minimal metabolic network of a reductive chemo-autotroph consists of 286 reactions yielding 287 distinct compounds (Srinivasan and Morowitz, 2009a; see also Srinivasan and Morowitz, 2009b). Without 'external' catalysis, none of the molecules of the rTCA cycle has been proven to progress in a cyclic fashion. The problem is recognised by Smith and coworkers.

The necessity of catalysis arises because in order for the cycle to progress, free energy hills must be overcome. Most reactions in the rTCA cycle in modern organisms are powered by the hydrolysis of a phosphate group of ATP, a moderately complex molecule that was unlikely to exist in considerable quantities in any environment on prebiotic Earth (Morowitz and Smith, 2007). However, ubiquitous energetic phosphates in hydrothermal vent systems (see also Martin *et al.*, 2008) might have provided the necessary energy to overcome the free energy hills (Morowitz and Smith, 2007), perhaps aided by small organic catalysts, transition metal sulphides (Cody *et al.*, 2004), or other present minerals. The entire mix of free floating small molecules in combination with mineral surfaces could in fact have aided in catalysis of the cycle (Copley *et al.*, 2007; and of subsequent pathways).

The most important assumption of the scenario is hence that in reducing primordial hydrothermal vent environments, the cycle will arise spontaneously (and the cycle will proceed self-amplifying; in a sense, the self-replicating behaviour of the cycle, i.e. self-replicating if the right catalysts are around, differs not that much from scenarios in which the entire cycle is truly autocatalytic and depends on the continuous supply of food molecules from the environment, at least if no change is considered). The assumption has not yet been proven: it is indeed the hypothesis to test. However, considering the lack of non-equilibrium thermodynamic theory (further elucidated in Morowitz and Smith, 2007), it is unlikely that the statement that life is like a 'lightning bolt' will be tested any time soon in a theoretical fashion. There appear to be a lot of 'problems' in the geosphere that could potentially be 'solved', but are not (e.g. the pile up of energy in terrestrial volcanic environments). Also, there may be many other solutions. A strong thermodynamic framework for non-equilibrium systems would provide the answer, but is lacking.

It appears that the only other way to test the hypothesis is experimentally: the theory predicts that in hydrothermal vent environments without life the rTCA cycle will spontaneously arise (perhaps followed by other metabolic pathways), thus these environments must be simulated. Obviously, simulating a geothermal environment at the bottom of the ocean is not the easiest of procedures, nor is it clear what kind of environments must exactly be simulated (considering e.g. that sea water chemistry in the time when life emerged was likely different from today), hence possibly the lack of any test tube evidence. In this regard the fabrication of a 'hydrothermal reactor' (Mielke *et al.*, 2010) is a very promising line of research, that might provide an answer to the hypothesis in the near future. In addition, as knowledge about catalysis by small organic molecules and mineral surfaces advances, crucial steps in the scenario may be elucidated.

The universality of metabolism and the anabolic core, although interesting in its own right, is no argument in favour of the hypothesis, but rather the restriction of contemporary life it is trying to explain. The other major explanation, common ancestry, is able to account just as well for it. However, the notion that thermodynamic ordering might rival Darwinian selection in explanatory power for complexity makes future investigation worthwhile, as will be discussed further on. The by Smith and coworkers recently deployed (and very clever) method of combining phylogenies with metabolic constraints on the ecosystem level in order to reconstruct the ancestral form of metabolism (Braakman and Smith, 2012a) does give credence to the suggestion that the rTCA cycle is a very ancient metabolic pathway for life, but it cannot by its nature distinct between accident and necessity, nor between metabolism- or replicator-first. The technique allows one to get a glimpse of the last common ancestor of contemporary life, but not of the origin of life, considering that the information is derived from contemporary genomes, as well as from modern carbon fixation pathways.

Hence, the universal anabolic core is no argument for an inevitable origin of life, and no

compelling evidence is available, but *if* part of complexity in contemporary life is indeed due to geochemical ordering, the molecules of the TCA cycle might be a good place to start looking for it (there are other suggestions, see Nitschke and Russell, 2009; Fuchs, 2011; Braakman and Smith, 2012b).

The extent of necessity

There is always a risk of misinterpreting analogies. The analogy with lightning bolts and hurricanes posed in the scenario is very prone to misinterpretation, because the relation between living and non-living things is foggy (if it were not, the origin of life would probably be solved already), and it implies a causation that is obscure. One confusing factor is that in order for life to 'solve free energy problems' in most of contemporary habitats, namely non-reducing non-hydrothermal vent environments, one most probably cannot escape the incorporation of history and evolution (also acknowledged by Smith and coworkers (e.g. Braakman and Smith, 2012b)). It is a legitimate and as yet unanswered question at which point necessity ends. Which part of contemporary life can be explained by geochemical organisation, and which by Darwinian selection, and how are the two related? It is not yet clear to what extent the analogy must be interpreted as truly comparable phenomena, and only a non-equilibrium theory for thermodynamics or experiments will provide the answer. However, one way to look at the analogy is the following:

In an early Earth geothermal environment, the only thing that may have been needed to get the rTCA cycle started was an easily accessible free energy source. Obviously, there should be enough electrons around for the cycle to progress in the first place; the notion that energy is required for life to exist appears extremely trivial. What is non-trivial, is that the cycle was the *only way of doing so*, just as a lightning bolt is the only way of overcoming charge differences in the atmosphere. In the initial stages of life, persistent order was forced by thermodynamics, leaving no room for variation. This does not necessarily translate into 'wherever there is an electron and carbon dioxide, there is inevitably the rTCA cycle' (although such a view may be adhered in a strong interpretation of the analogy), however it might mean that 'wherever there is an electron, the only way to harvest it is via the molecules of the cycle', and in the right environment, catalysts nearby, the cycle might have emerged spontaneously. This poses constraints on life: life is whatever you can make out of the molecules of the cycle. There is some variation possible in the harvesting mechanism, as is demonstrated by (very complex) electron harvesting pathways in modern photo-autotrophs, but the molecules of the rTCA cycle are and have always been strongly embedded in core metabolism. In order for life to persist, it can do many things (constraints at higher levels such as DNA and protein will be forced by Darwinian selection), but changing its anabolic core might not be one of them.

The view is very different from the more common held view, namely that universality in metabolism arose due to common descent. It defies Theodosius Dobzhansky's famous statement that 'nothing in biology makes sense, except in the light of evolution' (although originally meant as a manifest against the use of religious argument in science, the notion does emphasise the evolution-centred view of biology). The restricted metabolic network of today arose not by sampling a subset of pathways from the primordial soup through Darwinian selection, but was already restricted from the start: the absence of organic molecules in metabolism of modern living systems with a complexity comparable to core metabolites is not due to selection, but due to chemical restrictions. The most important statements the theory makes is that core metabolism *needed no significant innovation*, and that complex life would not be able to emergence on a different foundation.

The key question is of course whether merely chemistry without selection is sufficient to explain the complexity of core metabolism (e.g. Fernando and Rowe, 2007). It is worthwhile to address the question in research to come. It does not matter whether the rTCA cycle is imagined as the origin of life, or a different pathway (see Nitschke and Russell, 2009; and discussions in Fuchs, 2011; Braakman and Smith, 2012b). Even though the proposed answers may differ, the question is universal:

to what extent can complexity be the result of geochemical ordering?

The repertoire of catalysis by small organic molecules and mineral surfaces in geothermal environments seems to be a crucial factor, that as yet may be largely unexplored.

4. Conclusions

Even though the replicator-first hypothesis faces serious difficulties, the three metabolism-first scenarios discussed in this paper do not as yet seem to provide a more promising solution to the origin of life enigma. The metabolism-first approach has demonstrated that Darwinian selection of metabolic networks is far from evident. Also, metabolism-first thinking has led to the question to what extent complexity can be the result of geochemical ordering, however not yet to an answer. Metabolic complexity forced by thermodynamics may be just as unreal as a spontaneous emerging self-replicating RNA molecule. Note that the possibility that metabolic pathways very different from contemporary ones provided the building materials for the RNA world has not been addressed, however the underlying question remains the same. Based on the three hypotheses, the claim made by Robert Shapiro that '[s]uch theories provide a robust alternative to ideas based on a replicator' (Shapiro, 2000, p. 173) cannot be supported. Questions posed in the present paper may provide guidance for research to come.

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