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An Overlooked Riddle of Life's Origins: Energy-Dependent Nucleic Acid Unzipping

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Abstract. The imposing progress in understanding contemporary life forms on Earth and in manipulating them has not been matched by a comparable progress in understanding the origins of life. This paper argues that a crucial problem of unzipping of the double helix molecule of nucleic acid during its replication has been underrated, if not plainly overlooked, in the theories of life's origin and evolution. A model is presented of how evolution may have solved the problem in its early phase. Similar to several previous models, the model envisages the existence of a protocell, in which osmotic disbalance is being created by accumulation of synthetic products resulting in expansion and division of the protocell. Novel in the model is the presence in the protocell of a double-stranded nucleic acid, with each of its two strands being affixed by its 3′-terminus to the opposite sides of the membrane of a protocell. In the course of the protocell expansion, osmotic force is utilized to pull the two strands longitudinally in opposite directions, unzipping the helix and partitioning the strands between the two daughter protocells. The model is also being used as a background for arguments of why life need operate in cycles. Many formal models of life's origin and evolution have not taken into account the fact that logical possibility does not equal thermodynamic feasibility. A system of self-replication has to consist of both replicators and replicants.

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Introduction

In contrast to rapidly growing knowledge of extant life and of its evolution, progress in the elucidation of the origins of life has been amazingly slow. Yet the emergence of life on Earth "is a fundamental, perhaps *the* fundamental question of biology" (Delbrück 1978). The idea of the RNA world notwithstanding, Oparin's theory, formulated in 1924, may still dominate the thinking about origins. Miller's discovery in 1953 that amino acids can arise from a mixture of simple gases seems still to be, after half a century of experimental effort, the major landmark of presumed laboratory simulation of the first steps leading to life on Earth. What was true in 1970 still holds in 2003: central and difficult questions on the origin of life remain unanswered (Allen 1970).

The analyses of experimental failures and of apparently highly improbable, if not impossible, assumptions on which the unsuccessful experiments were based (e.g., Shapiro 1986; Thaxton et al. 1992; Chadwick 2001) have led to revival of Arrhenius' and Crick's conjecture that life on Earth may be of extraterrestrial origin or of Hoyle's hypothesis that life may have been created by an intelligence inherent to the universe. However, to account for the advent of life by special (divine) creation in the sense of traditional religions and mythologies, it is most

symptomatic that one of the former leading proponents of the "naturalistic" explanation of the origin of life, Dean H. Kenyon, is now speaking of "the impasse in current laboratory and theoretical research" in this field, expressing his "growing doubts that life on earth could have begun spontaneously by purely chemical and physical means," and favoring the conclusion that "it is fundamentally implausible that unassisted matter and energy organized themselves into living systems" (in Foreword to Thaxton et al. 1992).

Kenyon may be right in his claim that "there is a fundamental flaw in all current theories of the chemical origins of life." This implies that what is needed now, more urgently than additional experiments along the established lines, are new ideas, new hypotheses. This communication has been motivated by such a requirement. A problem which has been apparently largely overlooked in thinking about the origins, how nucleic acid unzipping had been managed in early phases of life's evolution, has been taken up. A tentative model of a primordial unzipping mechanism is being considered. Focusing on this specific question may entail a change of the whole perspective.

Elaboration of Conceptions

Replicators and Replicants

Experimental scientists can often do without explicit and precise definition of concepts. On the other hand, meaningful scientific discourse and intellectual satisfaction from understanding of relevant features of the world seem to rely upon the clear-cut definitions.

Life has generally escaped a straightforward definition. As pointed out by Maynard Smith and Szathmáry (1995, p. 17) there are essentially two classes of definitions of life. By the phenotypic definition, a thing is alive if it has parts, or "organs" which perform functions. Another definition, hereditary, defines life as any entity that has the property of multiplication, variation, and heredity. The latter definition implies the notions of replication and replicator. These notions have also diverse definitions. According to the opinion of Morowitz et al. (1988), which is relevant to the reasoning of the present paper, "replication is defined as any energy-requiring growth process in which an organized assembly of molecules produces similar assemblies over time. We do not require sequence-mediated informational transfer, nor a precise doubling of the assemblies." On the other hand, by the term replicator is usually meant not an assembly, but a distinct entity that is implicated in the sequence-mediated information transfer. The concept of replicator, popularized by Richard Dawkins as a genetically active unit whose structure is copied repeatedly (the gene, one kind of replicator, being a segment of a molecule of DNA), has often been criticized as being rather vague or even rejected as a misnomer (Ghiselin 1987; Vaneechoutte 1988). It has been argued that the suffix -or refers to an active agent, a processor. But

> current DNA-genes are nothing but a material instantiation of information on how to make RNA and proteins, and perform no replication. Genes are replicated informational macromolecules, unable to replicate on their own, … only the system cell as a whole enables self-replication. ... 'Replicata' or 'replicates' ('that what is/can be replicated') might be a more general term for informational molecules (nucleotide genes) or cultural bits of information (memes) which are replicated. (Vaneechoutte 1998)

Many would agree. Dover (1999) expresses the view, which most biologists may be intuitively aware of, that "the only known biological entity capable of autonomous replication is the cell." Atlan (1999) refers to Lewontin (1992) that DNA is a dead molecule, one of the least reactive, with no capacity to reproduce itself. It needs proteins to be reproduced. DNA does not fabricate proteins, as has often been said but, if fact, there are proteins that fabricate DNA.

The term "replicator" is so deep-rooted in the current biological discourse that it would be vain, and perhaps also misleading, to try to modify or to replace it. Yet to avoid confusion, the passive character of replicators should be made explicit. The term "replicator" may be complemented with another term, the "replicant," designating an active agent, which alone, or in association with other agents, is doing the work of replicating the replicator. And, indeed, self-replication would be the process that can only be accomplished by the system containing both replicator and replicant. This idea will be substantiated in the next section.

Already more than 30 years ago, Allen (1970) came up with the claim that "dogmatic insistence on the need for linear polymers or 'informational macromolecules' as a basis for life may be a consequence of focusing on the mode of reproduction in modern organisms instead of on the elementary requirements of natural selection." He hypothesized that "the first regular self-replication of ordered linear polymers on the earth was preceded by a period of evolution by natural selection among simpler organic molecules that did not serve as templates but reproduced by promoting other reactions critical for their own synthesis." Allen proposed that instead of self-replication and self-reproduction, this minimal requirement could be characterized as *self-dependent multiplication*.

A number of investigators came up later with similar scenarios. They all may imply that "non-genetic information exists in metabolic functions and probably preceded genetic information historically" (Root-Bernstein and Dillon 1997). Kauffman (1993, p 285) has provided convincing arguments that "molecular systems, in principle, can both reproduce and evolve without having a genome in the familiar sense of a template-replicating molecular species" (see also Lee et al. 1997). A more specific self-organization should be mentioned, which does not only involve a set of cross-interacting chemical reactions in a homogenous phase, but also organization in space due to capacity of amphiphilic molecules to spontaneously assemble into structures (e.g., Bachman et al. 1992; Walde et al. 1994; Pohorille et al. 1996). Segré et al. (2001) showed both by computer modeling and experimentally that mutually catalytic sets of simple organic molecules form assemblies exhibiting a significant degree of homeostasis and are capable of self-replication and rudimentary chemical evolution.

The analysis of genome evolution, carried out by Woese (1998, 2002), and its implication may be most revealing in this respect. Descending the evolutionary ladder down to the simplest life forms, Woese showed how species individuality is becoming more and more vague, blurred, until, at the bottom, the universal ancestor appeared—not as a discrete entity but, rather, a diverse community of cells that survives and evolves as a biological unit. According to Woese, in this early phase of biological evolution, cellular entities ("progenotes") were very simple, information-processing systems were inaccurate and mutation rates and horizontal gene transfer were elevated. Woese has used impressive metaphors of "genetic temperature" and of "genetic annealing." At the beginning, the "genetic temperature" was very high, preventing formation of more complex and stable structures.

If we extrapolate Woese's reasoning to still earlier phases of biological evolution, if we go in the "evolutionary annealing" process backward in time, up to the very origins of life on Earth, we witness the "genetic temperature" still rising, the evolving systems being still more fluid (and even "gaseous") and intermixed—indeed, we watch the flames of the primordial "evolutionary fire," from which everything living derived. Not only are the boundaries between evolving systems becoming indistinct, but also the notion of life itself is becoming blurred and the question about the definite instant of its beginning meaningless.

It is then logical to visualize that, in the forward course of "evolutionary annealing," chemical processes, permitted by thermodynamic and kinetic contingencies, were running independently and concomitantly: prebiotic syntheses of amino acids, nucleic acid bases, sugars, lipids; their polycondensations and self-organizations. Proteins and nucleic acids may have evolved in parallel, rather than sequentially. The latter view is preferred mainly by those who adhere to the hypothesis of the "RNA world" ("RNA first" [e.g., Joyce 1989; Poole et al. 1998]; different views, essentially "metabolism first," have been presented, for instance, by Wächtershäuser [1990]; De Duve [1991]; Dyson [1999]; and Lahav [1999]; the recurrent controversy has been analyzed recently by Wills and Bada [2000]). The very contingencies determined the evolutionary instants at which the various independent pathways associated to form novel, integrated entities. The association of nucleic acids and proteins may have given rise to the present-day form of cellular life, in which permanence is being assured by the collaborating DNA replicator–protein replicant systems. But the temporal permanence of nongenic patterns continues and manifests itself in the form of epigenetic inheritance (Maynard Smith 1990) and possibly as temporal continuity of membranes (Poyton 1983). The corresponding replicators and replicants may await to be given their names.

Living Systems Are Machines Operating in Cycles

Any definition of life expresses, explicitly or implicitly, the tendency of the living system to self-preservation, to permanence, to onticity. This applies also to replicators and replicants, whether viewed as systems for themselves—within the conceptual framework of "selfish-genery" (the name used by Dover 1999)—or, in a complementary conception, as tools in service of higher-level systems. Maintaining persistence of the living system in the changing world is a specific kind of work, the "*ontic work*," done by the system both on itself and on its environment. In this sense, living systems are machines, or engines. To do work permanently, a machine must work in cycles (Fenn 1982). Upon accomplishing some operations, coupled at distinct steps to input and output of energy, it must return to the original state and only then can it start another cycle of work. Replication, a common strategy of living systems to maintain their onticity, can be conceived of, at a definite level of graining, as one cycle of ontic work.

Chemical cycles, of which life abounds, may have evolved to allow a subtype of ontic work that can be dubbed the "*chemical ontic work*": chemical compounds, which left alone are thermodynamically or kinetically unstable, keep their permanence by being members of cycles. ATP is surely the best example of such a compound. Such cycles may have been of prime importance in prebiotic chemistry by keeping labile substrates in plentious supply.

To make the ontic work most efficient, the living system has also to do another kind of work, the "*epistemic work*": to record properties of the environment, to evaluate the records, and to decide how to act appropriately. Living systems are cognitive systems. The epistemic work of living beings has much in common with the work of computers. It has been shown by Kuhn (1988) that molecular replication itself has some features similar to the operation of a computer. Complementary pairing of nucleic acid bases is a spontaneous process, since the free energy of the system is decreasing. Thus, the resulting double helix of a nucleic acid molecule is in a state of minimum free energy. However, in order to start another pairing, the double helix must be unwounded and this requires input of energy. Kuhn (1972), and, similarly, Anderson (1983) have assumed that the cycling of successive pairing of bases to form a double helix, followed by unwinding and separation of two single strands, was made possible early in evolution by periodic heating and cooling of the environment (such as the day–night change). Interestingly, Weiss (1981), who took up the possibility of self-multiplication of layers of silicates as an inorganic model of biological self-replication, was also aware of the necessity of work in cycle to render continual replication possible. He thought that conditions may be provided by cycles of thawing or by the change from rain to drought.

Generally, however, the essential fact that the replication of nucleic acid involves not only ordering of complementary nucleotides along the chain of the polymer molecule and successive ligation—a process that is thermodynamically spontaneous—but also energy-dependent separation of the two helices, has been overlooked or underrated. Even those who may have been aware of the problem and proposed thermal denaturing of complementary strands as a primordial mechanism may have not envisaged of how to get the separated strands far apart to avoid self-annealing that would prevent complementary resynthesis and copying of the strands. In a review of facts and speculations about the origin of life (Orgel 1998), an optimistic scenario pictures the synthesis of primordial RNA, the main actor of the presumed RNA world. It is depicted how copying of longer template RNAs, using monomers or short oligomers as substrates, leads to the accumulation of a library of double-stranded (dsRNA) molecules. "Finally, an RNA double helix, one of whose strands has generalized RNA-polymerase activity, dissociates; the polymerase strand copies the first to produce a second complement—and so on." This last sentence is added almost in passing, as if thermodynamic obstacles did not make such a concourse of events virtually impossible.

The point is that a single molecule cannot be both template and replicase at the same time. Let us admit the temperature cycling as external conditions. In the phase of high temperature the dsRNA would be unwounded, denatured, but then the ribozyme would be denatured as well and could not function as replicase. Even if it remained native and active, how can dsRNA be formed if the conditions continue to be denaturing and *vice versa*?

In a recent paper with an ambitious title "Synthesizing Life," Szostak et al. (2001) envisaged a lipid vesicle, which would contain an encapsulated ribozyme functioning as replicase, to be a simple protocell that "would be nearly, but not quite, alive." To vivify it, Darwinian selection should be introduced, for instance, by having in the protocells, along with the replicase, a ribozyme that would synthesize amphiphilic lipids and so enable the membrane to grow. The membrane and the genome would then be coupled and the "organism" as a whole would "be fully alive and evolve." However, once the issue of strand separation during or after replication is taken into account, the simple scenario no longer works. The authors had to admit that this would imply more complexity in the RNA replication machinery. It would require an additional ribozyme functioning as RNA helicase to carry out energy-dependent strand separation. Other alternatives, such as helicase activity as a portion of the replicase or replication by strand displacement on a duplex template, would also introduce additional complexity.

The necessity of energy cycling has been overlooked in many published models of molecular self-replication, which otherwise may have been mathematically well founded and/or functioning in a computer populated with creatures of the automata theory (Von Neumann 1966). Logical possibility does not equal thermodynamic feasibility. Count Münchhausen cannot pull himself up from a swamp by his own bootstraps. A replicator cannot be simultaneously a replicant.

Helicases are doing the work of separating double-stranded DNA or RNA into two single strands in contemporary organisms (Borowiec 1996; Von Hippel and Delagoutte 2001). They are in no way simple catalysts speeding up thermodynamically spontaneous reactions. They function as molecular engines and use free energy of nucleotidetriphosphate hydrolysis to accomplish this uphill task. Helicases participate in all aspects of DNA metabolism, including replication, recombination, repair, and transcription. An analysis of the genome of the yeast *S. cerevisiae* showed that about 2% of its genes (at least 134 genes) encodes helicases (Egelman 2001). Even in a most simple system of *in vitro* replication, which constitutes a step in a procedure of isothermal amplification of nucleic acids (Guatelli et al. 1990), one of three enzymes used, T7 RNA polymerase, has to do unwinding work. This has been recently docu-

mented by a detailed study of the structure of the enzyme (Tahirov et al. 2002).

The problem, essentially identical to that of double-strand unwinding, of how to accomplish a full work cycle by getting out of the minimum of free energy, is common to all attempts at molecular self-replication in the absence of enzymes. In the laboratory, there has always been a human subject fulfilling the role of a "replicant" in the successful accomplishment of "self-replication" of oligonucleotides (Von Kiedrowski 1986), peptides (Lee et al. 1996), or abiological organic molecules (Winter and Rebek 1996). Humans are indispensable agents in *in vitro* evolution of artificial RNA polymerase ribozymes (Johnston et al. 2001) and function as "coreplicants" conjointly with the ribozymes. And, of course, unwinding of DNA is a daily business of researchers in molecular biology. Human investment of work is obviously necessary for all these manipulations.

No such "*deus ex machina*" was available in biological evolution. Biological evolution had to "search" for millions of years, possibly trying any accessible ways of how to solve one of the problems of molecular replication, until helicases were "invented." The present-day helicases can do their work because they carry in their structures embodied evolutionary knowledge. Humans can be many orders quicker, more versatile, and more successful, because they possess much of the knowledge that evolution was gathering in the course of more than 3 billion years.

If humans will soon succeed in designing full-fledged artificial life, and still face the enigma of the origins of "natural" life unresolved, we should not be surprised. The artificial life will likely be set up by applying a narrow set of physical principles (as considered by researchers on artificial life [e.g., Langton et al. 1992; Taylor and Jefferson 1994], while the natural life is based on chemistry. Not rigid constraints, but rather the very breadth of chemical possibilities may make the reproduction of the traversed evolutionary trajectory so difficult. An evolutionary option of a specific biochemical process, a transient one, no longer operating in contemporary cells, probably one of several possible, is being considered in the next section.

Model of Primordial Nucleic Acid Unzipping

The model is based on the ideas of those investigators who linked the emergence of life with the capacity of abiotically formed amphiphilic molecules to self-organize into lipid bilayers, which eventually formed closed vesicles, giving rise to "protocells" (Wilson and Lin 1980; Koch 1985; Cavalier-Smith 1987; Morowitz et al. 1988; Yanagawa et al. 1988; Walde et al. 1994; Pohorille et al. 1996; Deamer 1997; Dworkin et al. 2001; Segré et al. 2001). The external environment is a source of energy and nutrients, while the internal volume of the protocell provides a closed microenvironment in which directed chemical reactions can occur. (A variant with a chemiosmotic free energy generator can be easily incorporated without affecting the basic tenet of the model.) In accord with the arguments presented in the first section it is supposed that independent parallel evolution of different classes of chemicals was running in the early phases of life's origin, punctuated by their occasional associations in different combinations. Chemical details of these presumed synthetic pathways, unknown so far, are not relevant to the model. It is assumed that peptides — having the capacity to catalyze, with little specificity, various reactions — coevolved and coexisted with lipids. In addition to polypeptides, a polynucleotide in the form of a double-stranded molecule was also present in the protocell. Ligation of complementary nucleotidetriphosphates, which had been produced in the environment and imported into the protocell, was catalyzed by (a) peptide(s). Over this standard model, proposed and discussed previously by a number of investigators, a new feature is superimposed: the double-stranded nucleic acid molecule is affixed by its 3′-termini to the membrane. As in the other models, the protocell is assumed to exhibit polarity to allow for its appropriate division, perhaps by having lipids distributed in nonrandom patches. In the present model, this would also prevent slipping of the molecular anchor in the lipid "ocean." In the evolutionary phase, considered by the model, the nucleic acid had no coding function. The association provided a solution of the unwinding problem: there were no periodic changes of temperature, as hypothesized by some investigators mentioned above, nor was there an investment of chemical energy, as is the case of present-day helicases, but osmotic energy was used to achieve strand separations of the double-helix polynucleotide (Fig. 1).

How realistic is this model? The aspects shared with the models of other investigators, including the nontrivial problem of the polarity of the protocell, are omitted from the analysis, and only those specific to the model are discussed. Both physical (and chemical) feasibility and evolutionary plausibility are taken into account.

Empirical data for the feasibility of the model have been provided by direct measurements of interaction forces between complementary strands of a single DNA molecule. (For recent review of the procedures see Bockelmann et al. [2002].) According to Essevaz-Roulet et al. (1997) mechanical forces necessary for the unzipping of a single base pair of a long segment of DNA were in the range of 10–15 pN and were

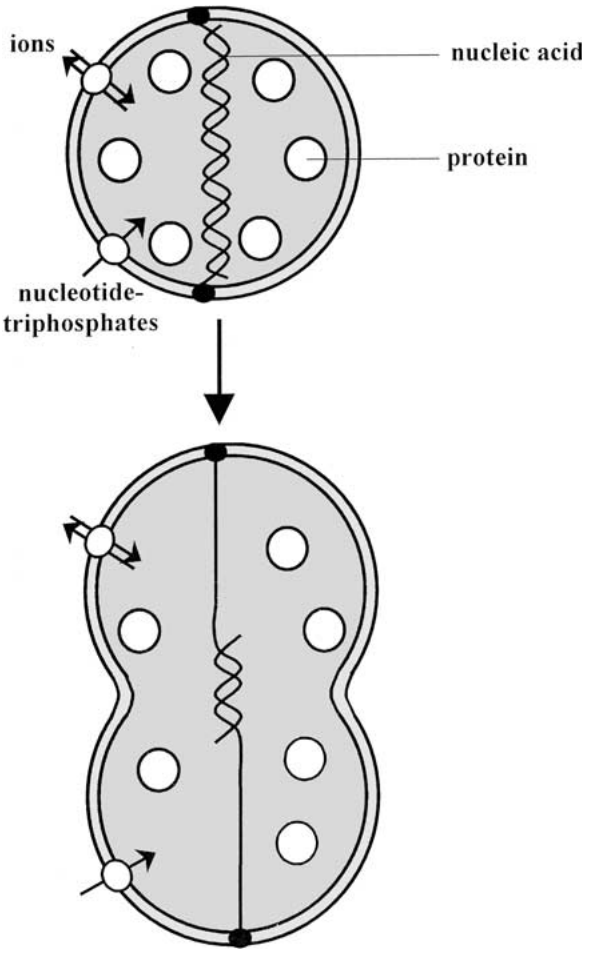

Fig. 1. A model of a protocell, with a molecule of nucleic acid affixed to the membrane, and of its division. The model embraces standard features considered previously by other investigators: a closed compartment filled with ions, amino acids, polypeptides, nucleotidetriphosphates, and their products and functioning as a chemical reactor; a self-assembling membrane composed of amphiphilic molecules and enclosing the compartment; carriers spanning the membrane and allowing import and export of ions, nutrients, and metabolic waste. Novel is the presence in the compartment of a double-stranded molecule of nucleic acid. The 3′-terminus of each of the two strands contains the sequence CCA and a hydrophobic amino acid (as in contemporary tRNAs), by which it is anchored in the membrane. Accumulation of synthetic products incapable of leaving the compartment creates osmotic forces which allow expansion of the membrane by intercalation of new amphiphilic constituents, but also pull the anchored strands of the nucleic acid longitudinally in opposite directions, causing unzipping of the double-stranded molecule.

sequence-dependent. Similar values of forces were obtained by Rief et al. (1999). These figures were lower than those found previously by Boland and Ratner (1995). Lee et al. (1994) measured forces involved in shearing apart opposite extremities of a 20-base DNA oligomer, composed of five repeating units. The magnitudes of the measured adhesive forces fell into four distinct populations of 1.52, 1.11, 0.83, and 0.48 nN, apparently corresponding to unwinding of 20, 16, 12, and 8 bp. According to Essevaz-Roulet et al. (1997), who tried to make the data of Lee et al. (1994) compatible with their own measurements, such high values of forces may include a strong coupling between bases because of the shearing motion, but may also be due to the fact that they were produced at a rather high unwinding rate. In any case, it seems likely—and this is crucial to the model presented here—that the force necessary to separate the strands by pulling each strand longitudinally in opposite directions is lower than the force that would be required to break the nucleic acid "zipper" straight by pulling it perpendicular to its length. A mechanical zipper shows this property.

Is osmotic force large enough to do the unzipping work? A polynucleotide containing 1000 bp would be 0.33 μm long, so it would span a protocell with a radius of 0.16 μm and a surface area of 0.32 μm^2. Its volume would be 1.7×10^{-17} dm^3; hence a 0.1 *M* solution in the protocell would contain 1 million molecules of solute. If the interior of the protocell contains low- and high-molecular-weight solutes in a total of 0.1 osmol $\cdot$ dm^{-3}, and the exterior 0.01 osmol $\cdot$ dm^{-3}, the osmotic force acting on the surface of the protocell would be 71 nN. This value is to be compared with the force of 10–15 pN necessary to "unzip" a single pair (Essevaz-Roulet et al. 1997). It appears that to consider osmotic force as *a* force taking part in the unzipping of the entire polynucleotide molecule—contributions of other forces, which take part in the expansion of the membrane (Koch 1985; Deamer 1997), are feasible—may not represent an impossible fancy.

Incidentally, the existence of "osmotic drive" has been proposed by Kauffman (1993, p. 338) in another context—to be involved in driving the synthesis of high molecular weight peptides or possibly RNA molecules. Kauffman maintains that if a protocell is placed in a hypertonic medium, the efflux of water, a product of the condensation reaction, should drive the reactions to the right, leading to the synthesis of larger polymers.

How plausible is the model from the evolutionary point of view? How and why should natural selection have favored the protocells carrying nucleic acid with no apparent function and, in addition, allowed the affixing of the useless molecule to the membrane by a mechanism that could hardly be set up in a single step? The following scenario may provide an answer: Before the mechanism of double-helix unwinding arose, all kinds of protocells, some with and some without nucleic acids, had thrived in a landscape decked out with products of essentially neutral evolution. If a molecule of nucleic acid had been present in a protocell, it had been carried over randomly into one of the daughter cells with no specific effect on fitness of the progeny. A chance "invention" of the simple mechanism of unzipping came up as one of the most fundamental in-

novations in biological evolution. Without it, the evolution would have been virtually stalled: there would have been just variations on the old theme. It opened the way for evolution of coding, transcription, and translation, but also for its own replacement by a more efficient mechanism involving helicase. The "invention" was not a one-step accomplishment. The anchorage of nucleic acid in the membrane brought along an "end-replication" problem, different from that faced by the contemporary linear DNAs (Kornberg and Baker 1992, pp 503–510), but similarly requiring additional, auxiliary chemical reactions. The major evolutionary feat may thus have been an outcome of long random tinkering in phases of neutral evolution. The importance of such a course of evolution has been documented by computer modeling in an impressive manner (Fontana and Schuster 1998; Forst 1998; Van Nimwegen et al. 1999).

It should be added that the principle of continuity does not necessarily imply that the primordial or intermediary stages of evolution should still be discernible in contemporary organisms: life, a universal tinkerer, is often throwing away obsolete constructions and procedures and removing any auxiliary scaffolds used, with no traces left. A single trace of the original machinery of unzipping may have been left by the supposed 3′-terminal fragment of the primordial nucleic acid, which in the model is used in anchoring the molecule to the membrane. To be able to bind hydrophobic amino acids it must resemble the 3′-terminus of contemporary tRNAs. Maizels and Weiner (1994) noticed that t-RNA-like structures are conserved in the replication of many extant genomes. The authors believe that tRNA-like structures arose very early in evolution and that their original function was to take part in replication and not in translation.

Conclusion

The present model, picturing a transient stage of the process of life's origin and evolution, does not differ from other available speculations in this domain of inquiry in being based on many unproved and possibly far-fetched assumptions. The advantage of the model is that its features can be tested experimentally. For instance, molecules of appropriately designed RNAs may be affixed to the membrane of self-reproducing liposomes (Walde et al. 1994), or *in vitro* RNA evolution may be targeted toward binding to membranes. The main virtue of the model may be a novel view on the origin of nucleic acid replication. The model is feasible, but the event may have not taken place: life on Earth, a chemical phenomenon, may have explored only a small region of the available space of possibilities offered by the breadth of chemistry. As a corollary of the model, the problem of molecular self-reference, underpinned thermodynamically, has come to the fore, with the implication that any self-replicating system must consist of both replicator(s) and replicant(s).

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