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ON THE EMERGENCE OF BIOLOGICAL COMPLEXITY: LIFE AS A KINETIC STATE OF MATTER

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Abstract. A kinetic model that attempts to further clarify the nature of biological complexification is presented. Its essence: reactions of replicating systems and those of regular chemical systems follow different selection rules leading to different patterns of chemical behavior. For regular chemical systems selection is fundamentally thermodynamic, whereas for replicating chemical systems selection is effectively kinetic. Building on an extension of the *kinetic stability* concept it is shown that complex replicators tend to be kinetically more stable than simple ones, leading to an on-going process of *kinetically-directed* complexification. The high kinetic stability of simple replicating assemblies such as phages, compared to the low kinetic stability of the assembly components, illustrates the complexification principle. The analysis suggests that living systems constitute a *kinetic state of matter,* as opposed to the traditional thermodynamic states that dominate the inanimate world, and reaffirms our view that life is a particular manifestation of replicative chemistry.

Keywords: biological complexification, chemical evolution, kinetic stability, kinetic state of matter, molecular replication, origin of life

1. Introduction

The process that led to the emergence of biological complexity – to life, remains highly contentious. Part of the uncertainty derives from the historic dimension to the problem. Uncovering the precise mechanistic pathway that led from inanimate to animate can in principle only be achieved by accessing and deciphering the historic record, for example, through being able to answer questions such as: *what were the conditions on the prebiotic earth, what might have been the first replicating system,* and so on. But a significant part of the uncertainty relates to the *ahistoric* nature of the physico-chemical processes that must have led to biological complexification. Here the question might be phrased as: *what general principles would induce matter to undergo a process of complexification in the biological direction*. As Wächtershäuser (1997) has commented, the science of chemistry is 'an ahistoric science striving for universal laws, independent of space and time or of geography and the calendar'.

Interestingly, though biological complexification is first and foremost a physico-chemical process, biology seems to have dealt more directly with the general principles governing that kind of complexification than chemistry. The evolutionary

process makes it quite clear that there has been a general tendency from less complex to more complex – from relatively simple prokaryote life forms to more complex eukaryote forms, and eventually to multi-cell life forms. The biological explanation for this process of complexification is of course Darwinian evolution – the operation of natural selection. In attempting to bridge between biology and chemistry, Eigen (1971) proposed that the very same process of Darwinian evolution that operated at the biological level must have initially also been operational at the prebiotic molecular level – a dramatic proposal that offered a conceptual unification of emergence with biological evolution. Yet despite that profound insight the nature of the link between chemistry and biology remains uncertain. Natural selection and survival of the fittest are biological terms and not part of the chemical lexicon, and a means of considering the process of complexification of inanimate matter in the biological direction in strictly physico-chemical terms would be desirable. Such a treatment should be able to explain not just the actual process of complexification, but also the striking characteristics that are associated with the living systems that emerged – the extraordinary degree of complexity, the far-from-equilibrium nature associated with such systems, and their teleonomic character – that sense of purpose that all living things display.

Finally, we think it quite unlikely that living systems represent a chemical pattern that is characteristic of just *one* particular set of so-called biomolecules. Presumably other molecular systems could also follow a process of complexification under appropriate conditions, leading to chemical systems that we would characterize as life. Tackling the issue of life's emergence in this more general fashion has the advantage of freeing us from many aspects of the perpetual debate surrounding the origin of life issue. Initially at least, we would not need to address the problematic historic-type questions, mentioned earlier. The purpose of this paper therefore is to attempt to further clarify the physico-chemical principles that underlie the extraordinary transformation of inanimate matter into simple life. In building up our model we utilize several concepts that are long part of the evolutionary debate – the primary role of replication, variation and selection, autocatalysis, catalysis, and self-assembly, but build these around the concept of kinetic stability and a general kinetic model of life that we have recently proposed (Pross, 2003; Pross and Khodorkovsky, 2004).

2. Discussion

In his classic book, *'What is Life?'* Schrödinger (1944) asks why atoms are so small? His answer is that it is not that atoms are so *small*, rather that we living entities are *big*. Life manifests the emergent properties of matter that has undergone an extraordinary degree of complexification. But what has induced this process of complexification? Or to rephrase the question more explicitly: (a) what kinds of molecules or molecular systems would be the likely starting materials for the emergence of chemical entities possessing life's characteristics, and (b)

what physico-chemical principles, when applied to those starting materials, would explain the process of complexification that lead to the emergence of life? In agreement with Kauffman (2000) we presume that the life phenomenon is likely to be a general one, that it reflects a fundamental property of matter, which, at least in principle, could be expressed in a variety of molecular systems, and not just the nucleic acid-protein based system that characterizes life as we know it. In Kauffman's words: 'A general biology awaits us'. We believe this assumption to be justified based on the general observation that the properties and characteristics of matter are invariably associated with *categories* of materials, and are not specific to one substance alone. Thus, for example, groups of substances exhibit particular group properties, e.g., the ability to crystallize, to conduct electricity, or to polymerize, *etc*. Indeed, in anticipation of the theme that we are about to develop, we can already note there is significant empirical evidence that *replicating molecules* form a category, and it is within this general category of molecules that the emergence of life problem can be usefully addressed. But our base line assumption differs from that of Kauffman in that we believe the known laws of physics and chemistry are sufficient for explaining the emergence of biological complexity – that the existing thermodynamic framework can accommodate living systems. Our perspective also provides a different response to a key question posed by both Kauffman (1993; 2000) and Dyson (1985): *Why is it that even the most minimal life is so complicated?*

2.1. BIOLOGICAL AND NON-BIOLOGICAL COMPLEXIFICATION

Given that the process of complexification in nature, at least as it pertains to *non-biological* systems, is a well-understood physico-chemical phenomenon, we first need to enquire if we can relate the process of *biological complexification* to its *non-biological* counterpart. Even cursory inspection of biological and non-biological complexification makes it clear that these two processes, though sharing common features, are actually quite distinct. Firstly, the level of complexity associated with biological complexification is of a totally different order compared to that associated with the 'normal' physical process of complexification. As Dawkins (1986) has written: *'We animals are the most complicated things in the known universe'.* But the nature of the two kinds of complexification is quite different because the *driving force* for each appears to be different. *Non-biological* complexification is explained by nature's global drive toward maximum entropy, or in the context of a closed system, as the drive toward lower free energy – toward the equilibrium state. The process can be *chemical* in nature, as manifest in the bonding between atoms to create molecules, or *physical* in nature, as exemplified by aggregation processes such as micelle or vesicle formation and crystallization. Biological complexification, however, though operating in a way that is of course consistent with thermodynamic principles, appears to be driven by some other 'force'. Biological complexification leads to *high energy, far-from-equilibrium* systems, rather than

the lower energy, equilibrium systems that are the target of non-biological complexification, so in that fundamental sense the two are quite distinct. Consequently the thermodynamic paradigm that treats biological systems as 'dissipative structures' (Prigogine, 1978; Weber *et al*., 1988; Peacocke, 1989; Thaxton *et al.*, 1984) and concludes that biological complexity *is* a manifestation of the Second Law of Thermodynamics, has been increasingly questioned (see for example: Corning and Kline, 1998a; Collier, 1988; Peacocke, 1989, pp. 216–7; Elitzur, 1994; Thaxton *et al*., 1984, pp. 151–2), and an alternative paradigm that has been developed over the past decade or so – that life is an emergent property of certain complex systems and that mixtures of biopolymers can spontaneously self-organize leading to the 'crystallization' of life (Kauffman, 1993, 2000; Segre *et al.*, 2000) – has also failed to muster general support (Lifson, 1997; Maynard Smith and Szathmáry, 1995).

2.2. Nature of biological complexification

We have recently proposed that the driving force for biological-like complexification is the kinetic power of autocatalysis operating on mutagenic chemical replicators (Pross, 2003; Pross and Khodorkovsky, 2004). All replicating systems are necessarily autocatalytic, and though autocatalysis is in no way inconsistent with the Second Law of Thermodynamics, its nature is such that it will most likely lead to *kinetically* favored replication products in preference to possible *thermodynamically* more stable ones. This approach led us to conclude that transformations involving *non-replicating chemical systems (i.e., regular chemical systems) and replicating chemical systems follow different selection rules.* The selection rule for the transformation of regular chemical systems is a thermodynamic one – *thermodynamically less stable* systems are converted into *thermodynamically more stable* ones. In contrast, the transformation of one replicating system into another follows a *kinetic* selection rule. Within replicator space – the space that (by definition) incorporates all replicating systems – transitions generally take place from systems that are *kinetically less stable* to ones that are *kinetically more stable.* So though *all* chemical reactions are governed by a combination of kinetic and thermodynamic factors, the relative importance of the kinetic factor in replicator space is such that a *qualitatively different pattern* of chemical behavior results. Thus we concluded that living systems exemplify a *kinetic state of matter,* and it is the distinction between kinetic states and the traditional thermodynamic states of matter that lead to living systems and inanimate matter exhibiting such strikingly different characteristics (Pross and Khodorkovsky, 2004).

Let us now apply this kinetic model to address the main issue of this paper: *how* and *why* does (mutagenic) replication and the process of kinetic selection in replicator space lead to a process of complexification? Or to phrase the question differently, if we assume the Eigen postulate (Eigen, 1971; 1992), that the process of emergence began with the appearance of some replicating molecule, *why would a one-molecule replicator tend to become a two-molecule replicating assembly*?

If we can demonstrate that such a process would tend to occur and would be governed by rules that are distinct to those that govern *thermodynamically-driven* self-assembly, then the kinetic approach may provide a basis for a more fundamental understanding of the general nature of biological-like complexification. That is indeed our thesis – that the emergence of life and its subsequent evolution involved the emergence of some mutagenic replicating molecular entity (the Eigen hypothesis), *and its kinetically-directed exploration of replicator space*.

2.3. General relationship between kinetic stability and complexity

Let us first consider in broad outline why the exploration of replicator space might lead to a process of complexification. We have already pointed out (Pross and Khodorkovsky, 2004) that the kinetically-directed exploration of replicator space seeks out *kinetically more stable* replicators (a kinetically stable replicator being defined as one that can maintain a significant equilibrium population of members under the existing environmental conditions). Since it can be readily demonstrated that complex replicators are kinetically more stable than simple ones (see below), the kinetically-directed exploration of replicator space could be expected to lead to a general process of complexification – from kinetically less stable replicators to kinetically more stable ones. Let us now explore this theme in more detail.

Inspection of the biosphere suggests that complex replicators are kinetically more stable than simple ones. The evidence, on this issue at least, is clear-cut. Current knowledge concerning simple molecular replicators indicates they are indeed kinetically unstable (Orgel, 1995). *In vitro* replication studies over the past two decades, such as those conducted by Orgel (1995) and von Kiedrowski (1986), have demonstrated that the process of molecular replication is a fragile one. Attempts to achieve non-enzymatic molecular replication have met with only limited success – the initial rate of replication is generally proportional to the square root of template concentration rather than to the concentration itself, due to the tendency of the template and its compliment to remain in (inactive) dimeric form. As a result autocatalytic template replication in the absence of an enzyme catalyst does not proceed to any great extent. A second difficulty, one that has been encountered with non-enzymatic oligonucleotide replication – the closest model for what might have been an early stage in the evolution of life – is that the complementary template that is initially formed in the replication process is never able itself to act as an effective template. Clearly effective replication cannot be induced in a system with that kind of constraint. Thus the general picture that emerges: non-enzymatic molecular replication, though feasible, is frustratingly problematic in a synthetic sense.

In comparison to simple replicators, complex replicators are stubbornly persistent, or in our terminology, kinetically stable. Cursory inspection of our planet makes that evident. Life on this planet abounds, and, as has been pointed out

by Gould (1996), bacterial life, in particular, is ubiquitous, having managed to establish itself in almost every conceivable ecological niche. Thus compared to simple replicators, which are manifestly fragile, complex replicators are robust and resilient, with some able to adapt to what would appear to be the most hostile of environments. This is, of course, true for all evolved life forms – from bacterial life through to multi-cellular life. Complex replicators are able to replicate successfully and prodigiously without the skillful guiding hand of an Orgel or a von Kiedrowski. The kinetic stability associated with all complex replicators appears to have derived directly from the process of complexification that has taken place. From this broad perspective therefore the emergence of life involved the exploration of replicator space beginning with simple, relatively (kinetically) unstable molecular replicators, and eventually extended to highly complex, robust and innovative, supramolecular replicating assemblies exhibiting high kinetic stability.

Before extending our argument to a mechanistic level, two points need to be made. First, the above mentioned relationship between kinetic stability and complexity does not apply to *biological* evolution, *but only to emergence*. An *Escherichia Coli* bacterium is *less* complex than a frog but *no less* kinetically stable – both readily maintain large equilibrium populations. Thus once kinetically stable *simple* life forms such as bacteria emerged, the on-going process of complexification associated with *biological* evolution did not necessarily involve a corresponding increase in kinetic stability. Both simple and complex life forms are readily sustainable; both manifest high kinetic stability. The diversity of life, *once it became established*, is associated with the on-going *divergent exploration of replicator space* seeking out additional kinetically stable entities (Pross and Khodorkovsky, 2004), rather than to any underlying change in the kinetic stability of resulting divergent species.

Second, our kinetic approach to the emergence of animate matter appears to open up a new perspective on the fundamental question first posed by Dyson (1985), and repeated by Kauffman (1993, 2000): *why is it that even minimal life is so complex?* Our argument would be that just as regular chemical space is mainly populated with entities of relatively high *thermodynamic* stability (highly unstable entities are normally formed only transiently, before their conversion to more stable entities), so replicator space is heavily populated with entities of relatively high *kinetic* stability. Kinetically unstable entities cannot be routinely observed in nature because they are – by definition – *kinetically unstable.* We hypothesize that the existence of kinetically *unstable* replicators would have been an essential, if transient, phase in the emergence of kinetically *stable* entities – an early stage in the kinetically-directed exploration of replicator space. Only replicators of minimal stability, already requiring a significant degree of complexity will be widespread. In other words, for a replicator to be sufficiently (kinetically) stable to be able to maintain a significant equilibrium population under whatever environmental conditions, it must achieve a relatively high, minimal level of complexity. This explanation differs from the one offered by Kauffman, who suggested that minimal

life is complicated because *life began complicated* – that the highly organized state associated with even the simplest living system can spontaneously 'crystallize' out of some complex mixture of random biopolymers. Difficulties with this approach have been discussed by Lifson (1997) and Maynard Smith and Szathmáry (1995).

So what molecular processes are actually taking place during complexification? A moment's consideration suggests that the physico-chemical manifestation of complexification is principally an ongoing process of *self-assembly*, though that self-assembly process is comprised of both static and dynamic components. The static component reflects the self-assembly of supramolecular entities from appropriate molecular constituents, as is evident in a variety of biological subsystems such as ribosomes, chromosomes and enzymes, and in entire biological entities, such as viruses and phages. The dynamic component is manifested by the emergence of reaction networks within the static assembly. Notice that within this general description of complexification the process of *compartmentation* does not need to be specifically addressed, since compartmentation is just a specific and specialized kind of self-assembly. So in order to better understand the process of complexification, we need to demonstrate that: (a) a simple modular molecular replicator would be driven to complexify into some minimal self-assembly, and (b) that such a minimal self-assembly would be kinetically more stable than the original molecular replicator from which it derived. Let us now examine whether the above assertions can be supported.

2.4. THE ROLE OF SELF-ASSEMBLY

The process of self-assembly, by definition, constitutes the exploration of chemical space. If however that exploration takes place within the context of *replicating* entities, then the exploration would be within replicator space, and, as noted above, selection within replicator space is kinetic rather than thermodynamic. We suggest that the relationship between self-assembly and the exploration of replicator space is a relatively simple one, and may be stated as follows: *If a molecular replicator acts as a catalyst for the formation of some molecule (or molecules) that self-assemble(s) with the replicator, thereby creating a molecular assembly of enhanced kinetic stability*, *then replicator sequences that facilitate the catalytic formation of such molecules will be kinetically selected. The result of that kinetically-directed process is likely to be the emergence of increasingly complex and increasingly kinetically stable replicating assemblies.* A process of kinetically-directed self-assembly reflecting the establishment of a symbiotic relationship between the assembly components, will have been initiated.

Is there any evidence for such a proposition? Actually the essence of kinetically-directed self-assembly and its importance to the replicative agenda are readily illustrated through one of the simpler biological examples of self-assembly – a bacteriophage. A typical phage is a supramolecular entity obtained from the self-assembly of a single nucleic acid molecule and several hundred protein molecules

(of one or several kinds) that encapsulate the nucleic acid within a protein coat. As separate entities the nucleic acid and any single protein molecule possess *low* (or zero) replicative capability under normal conditions, i.e., both components are kinetically *unstable*. In contrast, however, the *molecular assembly* under those same conditions manifests demonstrably *high* kinetic stability, as the assembly is capable of maintaining a large equilibrium phage population – phages are successful biological entities. Of course this high kinetic stability derives directly from the symbiotic interaction between the two components. The protein coat contributes to the high kinetic stability in two ways: first, it protects the encapsulated nucleic acid from chemical attack, and second, it is structured so that it can recognize the host bacterial cell membrane, allowing insertion of the nucleic acid into the cell, where it converts the bacterial cell into a phage-replicating factory. The nucleic acid contributes to the replicative agenda through its nucleotide sequence, not by being a better replicator in a direct kinetic sense, but through expression of the specific protein molecules, whose role in enabling nucleic acid replication to occur at all is critically important. Hence phage replication clearly demonstrates the crucial role that self-assembly plays in accessing elements of greater kinetic stability within replicator space – the process of complexification *has generated a kinetically stable molecular assembly from kinetically unstable molecular components.* In fact the nucleic acid - protein symbiotic relationship with its kinetic consequences is not just operational in phage replicative function, but permeates all of biology. It is clearly the most fundamental of the many symbiotic relationships that exist within living systems.

Of course the example of phage structure, as an element in replicator space, only applies in a biotic context where bacterial life is already widespread, which would not have been the case in prebiotic times. In that sense the phage structure provides no real evidence for the transitional structures between replicating molecule and replicating cell in the early stages of life's evolution. But the fundamental principle inherent in the phage replicative capability would be applicable at all times – biotic *and* prebiotic: *self-assembly that leads to enhanced kinetic stability under existing environmental conditions, whatever those might be – prebiotic or biotic – will be kinetically selected.* Such supramolecular entities together with their appropriate replicating microenvironments would constitute new and more stable elements in replicator space.

The phenomenon of replicative symbiosis through self-assembly leading to kinetically stable systems is well known in biology. Mitochondria and chloroplasts, essential cellular organelles within highly evolved eukaryotic cells, are now thought to have come about through the merging of a bacterium with a simple cell (Margulis, 1981). Such a process of *biological* self-assembly, termed endosymbiosis, though taken from the world of living systems, further demonstrates the principle of kinetically-directed complexification mentioned earlier. What we are saying is that *the principle of kinetically-directed self-assembly is general and applies to both biotic and prebiotic worlds.* Self-assembly of elements that are (or be-

come) replicatively coupled (Norris and Fishov, 2001), leading to kinetically more stable systems, constitute favorable transitions within replicator space. The above argument suggests that supramolecular aggregates that are formed by kinetically-directed self-assembly are distinct from the ones formed through non-biological thermodynamically-directed self-assembly. As we have written previously (Pross and Khodorkovsky, 2004), those aggregates formed by kinetically-directed self-assembly constitute a *kinetic state of matter*, as opposed to the thermodynamic states – solid, liquid, gas, which dominate the inanimate world. Not surprisingly, the characteristics of kinetically-directed aggregates exemplifying a kinetic state of matter differ dramatically from matter that has undergone thermodynamically-directed aggregation. An important test of this hypothesis will be to establish whether life's distinguishing characteristics – for example, its far-from-equilibrium character and its teleonomic nature – can be derived from its properties as a kinetic state. We will conduct this test regarding life's far-from-equilibrium character in Section 2.6. The connection between life as a kinetic state of matter and its teleonomic character is the subject of another paper (Pross, submitted).

2.5. THE ROLE OF MUTATION

We have discussed the importance of self-assembly in accessing entities of greater kinetic stability within replicator space, but the question arises how the particular molecular components that assemble with the replicator come about. The likelihood that a replicating molecule will find itself in the vicinity of the particular species with which it can successfully self assemble to form a kinetically more stable entity is remote. The highly specific structure of those molecules indicates that the appearance of such molecules must come about in some *directed* process. Indeed it is the molecular replicator, through its nucleotide sequence, that induces the appearance of the appropriate molecule(s) so that the process of self-assembly is actually a *programmed* one. In other words *the exploration of replicator space is initiated by the exploration of sequence space* – the space that incorporates all possible sequences of the replicating biopolymer. Let us elaborate on this theme.

Each and every nucleotide sequence constitutes a unique element in *sequence space* (Eigen, 1992, 2000). Imperfect replication of the modular autocatalyst is a primary means by which sequence change takes place. The pioneering work of Spiegelman (1967), Eigen (1992), Orgel (1992) and more recent work on ribozymal evolution (see, for example: Johnston *et al*., 2001; Joyce, 2002) have indeed confirmed that modular replicators can undergo mutation, selection, and evolution. But while mutation clearly leads to new elements in sequence space, that process does not, in itself, constitute complexification. It is the *indirect* effects of mutation – the generation of catalytic networks and aggregation through self-assembly, that are actually responsible for the trend toward complexity and life. So we now need to consider the general process by which mutation might initiate self-assembly and the emergence of catalytic networks.

Catalysis is central to all of life's processes, including the process of complexification, so in order to understand how complexification came about, we need to understand the means by which catalytic function emerged. The existence of ribozymes – RNA sequences exhibiting catalytic qualities, suggests how. Mutations resulting in sequences exhibiting replicase character would be kinetically selected over catalytically inert sequences. In other words more effective replicator sequences are not necessarily those that are faster replicators *per se*, but those that would exhibit catalytic (replicase) character, thereby enhancing replicating capability through *secondary* catalysis. Thus the exploration of replicator sequence space would preferentially lead to the selection of sequences exhibiting such catalytic capabilities.

But of course, the emergence of catalytic function need not be restricted to catalytic activity associated with the replicator itself. Broader catalytic function may emerge through a *co-evolutionary* process whereby the replicator and other catalytically generated products are formed. The co-evolution of oligonucleotides and oligopeptides first described by Eigen (1971) and Eigen and Schuster (1977), is now thought to exemplify this type of process (Lahav *et al.*, 2001; Kunin, 2000; Norris and Raine, 1998; Lahav and Nir, 1997). Thus autocatalytic oligonucleotides, X, may not only catalyze their own formation, as expressed in Equation 1:

$$A + B + C \xrightarrow{X} X + Z \tag{1}$$

$$L + M + N \xrightarrow{X} Y + Z \tag{2}$$

(where A, B and C are mononucleotides and Z is some by-product such as pyrophosphate), but may also catalyze the formation of other entities – for example, an oligopeptide, Y, as expressed in eq 2, (where L, M and N are amino acids) in what could be interpreted as the beginning of *coded catalysis*. After all, as Di Guilio (2003) has recently pointed out, the genetic code is just an elaborate form of coded catalysis, though the early mechanism for that coded catalysis, which eventually evolved into the highly complex ribosomal machinery of modern organisms, remains controversial. Thus, if the oligopeptide product once formed turns out to possess catalytic properties with respect to the replication reaction itself, then the beginnings of a catalytic network would be established. The net result of such a catalytic network would be increasingly effective *autocatalytic* (i.e., replicative) function. Replicators whose sequence is such that they would lead to the formation of *more* effective co-catalysts for the replication reaction – in effect primitive replicases – would be selected for. Indeed the recent discovery of a variety of autocatalytic networks – cross-catalytic, autocratic, and hypercyclic – demonstrates the versatility and generality associated with the emergence of catalytic capability (Lee *et al*., 1997). The process demonstrates the symbiotic power that catalytic networks can bestow on replicative processes as part of the ongoing kinetically-directed exploration of replicator space.

The significance of the replicator's tendency to elongate through asymmetric genetic drift (Pross, 2003) can now be pointed out. While Spiegelman's experiments (1967) on replicator mutation indicated that shorter replicators would be faster replicators, this particular experimental result did not demonstrate the catalytic potentialities of longer genomes. In an appropriate co-evolutionary environment, where nucleic acid replicators could bring about the catalytic formation of oligopeptides from available amino acid building blocks, it is evident that the catalytic potentiality of a shorter replicator would be much more limited than that of a longer one. For example, a 12 nucleotide long nucleic acid might only be able to catalyze the formation of a 4 amino acid unit long oligopeptide (12 divided by 3, the number of base pairs required to characterize a specific amino acid), for which there would be just 20^4 ($\sim 10^5$) possible oligopeptide sequences. For a 100 nucleotide long nucleic acid however, the number of possible 33 unit amino acid oligopeptide sequences would be 20^{33} ($\sim 10^{43}$). Clearly the catalytic potential hidden within a family of $\sim 10^{43}$extended oligopeptides would be enormously greater than that within a family of $\sim 10^5$shorter ones – for both structural and numerical reasons. This argument would apply equally to ribozymal activity that may well have preceded enzymatic activity – longer oligonucleotides would be more likely to exhibit effective replicase activity than shorter ones – again, for both structural and numerical reasons. Over time therefore the continual exploration of replicator space would lead to the evolution of *longer* genomes expressing increasingly efficient catalytic and autocatalytic networks, all incorporated within a supramolecular aggregate. Thus a cell may be though of as *a kinetically selected element within replicator space* in which each and every component of that complex system interacts symbiotically in order to create a more effective replicating entity. Replication of the *entire* entity takes place, directed by its genomic heart.

2.6. THE ROLE OF METABOLISM

We noted earlier that thermodynamics, which drives systems *toward* equilibrium, cannot have been the driving force for the emergence of *far-from-equilibrium* entities. The question then arises: how does our kinetic model explain the emergence of far-from-equilibrium replicators dependent on external energy resources?

We have previously noted that the exploration of replicator space, though consistent with the laws of thermodynamics, is driven by the kinetic power of autocatalysis. A most significant step in the process of complexification would have been one in which mutation, self-assembly and kinetic selection would have led to the incorporation of an energy gathering capability (for example, the assembly of a nucleic acid with a simple photoreceptor to create an energy absorbing replicating assembly). This specific act of self-assembly, resulting in the formation of an energy gathering system, would have constituted the first step in the exploration of replicator space *in the far-from-equilibrium regime.* Complexification, though potentially kinetically beneficial, is at some stage likely to become thermodynamically unfa-

vorable. However, the incorporation of an energy gathering metabolic system, *in itself a form of complexification,* enables the thermodynamic constraints associated with complexification to be overcome (Pross, 2004). Thus the *pre-metabolic* exploration of replicator space would have involved the *kinetically-directed* and *thermodynamically-driven* reactions of *non-equilibrium* systems, while the subsequent *metabolic* exploration of replicator space involves the *kinetically-directed and* (effectively*) kinetically-driven* reactions of *far-from-equilibrium* systems. Simply, the incorporation of an energy gathering capability, which itself comes about through kinetic selection, enables the region of replicator space that contains complex, far-from-equilibrium replicators to be accessed. In an evolutionary sense this metabolic development was a crucial one since, as we have already discussed, a fundamental characteristic of replicator space is that *complex, far-from-equilibrium replicators tend to be kinetically more stable (i.e., fitter) than simple, non-equilibrium ones.* Accordingly, energy-supplying metabolic systems would facilitate a far-reaching structural exploration of replicator space, while satisfying the inherent thermodynamic constraints associated with ever-growing complexity. Through the emergence of energy-gathering metabolic systems – either chemically or photochemically based – nature has discovered that it is possible to have one's cake and eat it.

In sum, the modern cell is the product of a long evolutionary process, whose intermediate protocell steps are not easy to identify, but the principle of *kinetically-directed self-assembly*, and the replicative advantages that such self-assembly would have afforded, would have characterized all of those intermediate stages. All are part of the evolutionary process – the exploration of replicator space. In addition we see that autocatalyst molecular mutation (exploration of sequence space) is the primary means of exploring replicator space, not so much through its direct kinetic benefits, but mainly through its secondary effects – the kinetically-selected incorporation of catalytic networks and self-assembly patterns that characterizes complexification. Thus complexification may be viewed as a multi-dimensional exploration of replicator space, one in which all possible degrees of freedom – material, spatial and temporal are explored, all part of the on-going search for kinetically stable replicators.

3. Concluding Remarks

The centuries old debate concerning the mechanism by which life emerged from inanimate matter persists. In this paper we have attempted to demonstrate that despite the many mechanistic questions that still await more definitive resolution, the conceptual mystery that has surrounded the emergence of life issue may be finally lifting. There is growing support for the idea that life is not just a complex set of chemical reactions that emerged by some, as yet to be discovered, historic mechanism. Life is first and foremost *a physico-chemical phenomenon,* and, as such, an-

imate and inanimate worlds need to be bridged in a fundamental physico-chemical sense. In fact it could be argued that such physico-chemical understanding should, at the very least, accompany, if not precede, detailed mechanistic understanding. So even though the precise mechanistic path from inanimate to animate remains shrouded in uncertainty, we believe the fundamental physico-chemical relationship between life and non-life may be characterized with increasing confidence. Our message is that the relationship between inanimate and animate matter is most simply reflected in the different selection rules that operate within the two parallel worlds of 'regular' and replicative chemistry. Elements within the non-replicative region of general chemical space – the domain of regular chemistry – are connected through the operation of standard kinetic and thermodynamic principles that direct systems toward lower free energy states. Accordingly, the primary directive within that space is *thermodynamic*. Elements of replicator space, however, being replicative in character (by definition), and therefore driven by the enormous kinetic power of autocatalysis, tend from kinetically less stable to kinetically more stable, i.e., selection in this space is effectively *kinetic.* It is process of kinetic selection that induces complexification, by enabling symbiotic interactions between components of the complexifying entity to take place, leading to replicating systems of greater kinetic stability – to supramolecular systems that manifest a kinetic state of matter. Thus replicative symbiosis plays a key role in biological complexification and this would be true at *all* levels of complexity given that the kinetic nature of the replicative process applies at every level of complexity – from single molecular entities, through simple molecular assemblies, via the complex assemblies found in single-cell life, and finally through to multi-cellular life. The process of complexification leads over time to the emergence of *thermodynamically unstable,* but *kinetically stable*, far-from-equilibrium entities whose predisposition is (almost) entirely replicative.

Our conceptual physico-chemical approach implies that life is not merely a characteristic of specific biopolymers. Life, as we know it, based primarily on nucleic acids and proteins, is now widely believed to have resulted from the *de novo* appearance of some particular replicating entity, possibly RNA-like (Gilbert, 1986; Joyce, 2002), but whose precise identity is not crucial to our conceptual understanding. Once such a modular autocatalyst happened to appear in an appropriate environment in which the imperfect replication reaction could take place, an element in replicator space was occupied, and at that point the natural process of evolution – the exploration of replicator space leading eventually to living systems – began. We would argue therefore, following some aspects of Kauffman's thinking, that other modular replicators, not just nucleic acid replicators, might also evolve into life given appropriate circumstances and conditions. One might even argue that the characteristics of life based on different modular autocatalysts would not vary significantly. Just as a television set can function using different components that perform the same task (e.g., electronic tubes or transistors), so living systems might be expected to exhibit similar characteristics based on evol-

ution through natural selection, *regardless* of whether the primal replicator was an RNA-like molecule, or some quite different modular replicator. Recent work on replicating systems (Eigen, 1992; Orgel, 1992; Joyce, 1994; Sievers and von Kiedrowski, 1994; Rebek, 1994; Lee *at al.*, 1996; Robertson *et al.*, 2000) makes it increasingly clear that the ability to self-replicate, though not a characteristic of a wide range of chemical systems, is an inherent property of a certain class of chemical systems. We suggest therefore that by defining the physico-chemical relationship between inanimate and animate matter in this more general fashion, a more fundamental understanding of the life phenomenon is obtained.

How do our ideas relate to Darwinian principles? Though Darwin explicitly excluded the problem of the origin of life from his evolutionary thinking, it is striking just how relevant Darwin's biologically formulated evolutionary ideas are, when considered in relation to the problem of emergence and its associated chemical context. His understanding of the crucial role of Malthusian exponential population growth in driving evolution anticipated the kinetic implications of chemical autocatalysis and its cardinal role in emergence. Common descent – Darwin's revolutionary proposal that *'all the organic beings which have ever lived on this earth have descended from some one primordial form'* (Darwin, 1859, p. 484), takes on prophetic character in the light of current ideas on the RNA world and molecular replicators. Indeed the essence of both this and earlier work is to build on established Darwinian concepts, that are traditionally biological in their context, and extend them into a wider physico-chemical arena. In so doing we find that Darwin's biological concepts of *natural selection* and *fitness* broadly translate into the key chemical concepts of *kinetic selection* and *kinetic stability*, and, that a simple reordering of the Darwinian principle: *living things replicate, and therefore evolve,* so as to read: *certain replicating things can evolve, and may therefore become living (if they are not so already),* is able to provide a simple conceptual bridge between inanimate matter and life (Pross, 2003). It is indeed remarkable that Darwin's central ideas, almost 150 years after the publication of the *Origin,* remain as relevant as ever.

Notwithstanding the progress that has been made, some difficult issues remain. Our treatment has not addressed the error catastrophe issue and how that would impact on simple assemblies, nor given any indication regarding the manner in which early self-assembly and first reaction networks would have become integrated into more complex assemblies. While molecular evolution in a test tube was demonstrated by Spiegelman (1967) some 35 years ago, persistent enzyme-free molecular replication, even under contrived laboratory conditions, remains elusive. Certainly no experimental evidence for kinetically-directed self-assembly – symbiotic complexification at the simple molecular level of the kind we hypothesize here – has been observed to date.

In closing we note that even though the key role of replication in emergence and evolution are now widely acknowledged, it is nevertheless striking that studies that consider life to be unrelated to, or only marginally related to, replication continue

to appear (e.g., Chaisson, 2001). Clearly the struggle to define the most elementary of concepts regarding life and its emergence continues unabated. In this paper we reaffirm the view that replication is the very essence of life (Pross, 2003), that life is an extended evolutionary manifestation of the physico-chemical process of replication, one that is capable of inducing a kinetic state of matter through a process of selection – autocatalytically driven kinetic selection – as distinct from the more general thermodynamic selection that governs ubiquitous inanimate behavior. Some 350 years ago van Helmont (1648) daringly declared: 'All life is chemistry'. As a possible update to that extraordinary insight, we would suggest a minor but significant qualification: 'All life is *replicative* chemistry'.

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