Review

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Why Is Abiogenesis Such a Tough Nut to Crack?

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Abstract: The latest life-origin literature is reviewed, along with grouping and classification of the most enduring models of abiogenesis. New trends are identified in origin-of-life thought. Astrobiology continues to deal mostly with inorganic and very elementary organic reactions in extreme environments. Nonequilibrium thermodynamics has received a great deal of attention. Attempts have been made to relate Bejan's Constructal Law to life and abiogenesis. RNA World preclusions only seem to have grown, while ribozyme/peptide models have expanded. The biosemiotic movement and code biology is gaining influence in refining a more complete description of life. Prof James Tour at Rice University in a fourteen-lecture series lists a number of synthetic biochemical hurtles remaining that still need to be overcome. But the need for some sort of informational recipe, instruction, steering and control now seems to be the most prominent area of focus and concern. Even a protometabolism needed active selection and controls rather than mere constraints or law. The latest literature still does not elucidate how protometabolism was orchestrated in an inanimate, pre-evolutionary environment.

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1. Introduction

This paper reviews the longest still-standing abiogenic models. It will also update the reader on the latest abiogenesis literature. Hundreds of papers have been published detailing *possible* historical scenarios that *might* have contributed to abiogenesis. Phrases like "could have," "are believed to have been," "perhaps," "possibly, "may have," etc. prevail. Carefully designed and controlled experiments affirm the possibility of certain critical "prebiotic" reactions occurring. The design and engineering outlined in Materials and Methods, however, often betray the contention of prebiotic plausibility. Empirical evidence of formal integration of these potentially prebiotic reactions into anything close to holistic metabolomics is virtually nonexistent.

Life-origin literature is also filled with subconscious teleological explanatory phrases such as "in order to . . ." and "so that . . ." which grossly violate the goal-less nature of evolution. Molecular evolution would have required active selection prior to the realization of any utility. It remains to be seen how sophisticated functionality, conceptually complex (not just complex) biosystems and homeostatic protometabolism could have arisen in an inanimate environment. The latest literature is examined in search of any new insights into the above conundrums.

Superb synthetic chemist, materials scientist, and nanotechnologist Prof. James Tour at Rice University has enumerated in a fourteen-lecture series the many synthetic biochemical hurdles that still need to be overcome within abiogenesis research for mere "possibility" to become "scientifically plausible."

2. The Most Enduring Abiogenic Models

What are the most long-standing models in the literature that have persisted into the last two to three years of peer-reviewed publication? Such models are by now quite well-developed and refined. They are certainly worthy of open-minded, honest consideration.

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Hundreds of papers leading up to the present emanate from four main categories of models: 1) Inorganic, 2) Organic Composomal, 3) RNA World, 4) Co-evolution models and 5) Informational models emphasizing the need for steering and controls rather than mere laws and constraints.

Table 1. Mature abiogenic models tend to fall into five main categories of thought.

Inorganic	Organic Composomal	RNA World	Co-Evolutional	Prescriptively Informational
Often involves transient-state metal ion reactions	Carbon chemistry more adaptable to current life	Self-replicating RNA instructed nucleic acid	Biochemistry is linked to the emergence of code and translation	Offers steering, orchestration & control
Offers spontaneous reaction occurrences	Offers spontaneous reaction occurrences	No explanation for initial source of instructions	Offers a possible reason for spontaneous "success"	Source of formal instruction is not clear
No directionality toward usefulness or metabolic success	No directionality toward usefulness or metabolic success	Self-replication function is not metabolic function	Provides some happenstantial directionality	Provides directionality toward metabolic success.
No plausible sustaining mechanism	No plausible sustaining mechanism	Properly programmed RNA could prescribe reactions	Directed chemistry could sustain "helpful" reactions	Instructions provide for sustained existence, functional productivity and heritability
Cannot build on any "successes"	Cannot build on any "successes"	Initial RNA instructions could mutate and evolve	Once coded instructions are "memorized," optimization could evolve	Algorithmic optimization becomes possible, even pursued
No heritability	No heritability	Some heritability inherent in self-replication	Heritability possible	Code biology could prescribe heritability of biofunction & biosystems

2.1. Inorganic Models Involving Mostly Transient-State Metal Ion Reactions

One of the first inorganic models was that of Cairns-Smith [1–6]. He proposed that life began as structural patterns in clays which self-replicated during cycles of crystal growth and fragmentation. This model died out decades ago. But as recently as 2022, Kloprogge and Hartman [7] reemphasized the possible role of clays in life origin. Kloprogge and Hartman argue that the data from Mars suggest that Fe-clays such as nontronite, ferrous saponites, and several other clays were formed on early Mars when it had sufficient water. They point out the recent finding of a chiral amino acid (isovaline) forming on the surface of a clay mineral on several carbonaceous chondrites.

As early as 1994, H, Russell and later Martin [8–10] attempted in an early geochemistry model to envision physical compartmentation from the environment as a substitute for present-day cells, cell membranes and cell walls. Their focus was on self-contained redox reactions involving inorganic matter. They initially proposed that life evolved in structured iron monosulphide precipitates in a seepage site of a hydrothermal mound. They believed a redox, pH and temperature gradient existed between sulphide-rich hydrothermal fluid and iron(II)-containing waters of the Hadean ocean floor.

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FeS and NiS can catalyze the synthesis of the acetyl-methylsulphide from carbon monoxide and methylsulphide which are constituents of hydrothermal fluid. The authors suggested a pre-biotic synthesis occurred at the inner surfaces of these metal-sulphide-walled compartments. The model proposes that these compartments restrained reacted products from diffusion into the ocean, providing sufficient concentrations of reactants to forge the transition from geochemistry to biochemistry.

Through time, Martin and Russell [11–17] believed that RNA-world chemistry could have taken place within these naturally forming, catalytic walled compartments to give rise to replicating systems. Sufficient concentrations of precursors to support replication would have been synthesized in situ geochemically and biogeochemically, with FeS (and NiS) centers playing the central catalytic role. They inferred that the universal ancestor was not a free-living cell, but rather was confined to the naturally chemi-osmotic FeS compartments within which the synthesis of its constituents occurred. The first free-living cells were suggested to have been eubacterial and archaebacterial chemoautotrophs that emerged more than 3.8 Gyr ago from their inorganic confines.

Others have contributed to this line of thought with modifications along the way [8–41].

Russell, Martin have argued that proton and thermal gradients at hot alkaline (pH 9-11) under-sea hydrothermal vents may have constituted the first sources of energy for abiogenesis. But RNA is extremely unstable at alkaline pH [42]. Such vents would be an unlikely location for RNA World evolution [43].

More currently, Liu et al [44] suggest that life is an out-of-equilibrium system sustained by a continuous supply of energy. In extant biology, the generation of the primary energy currency is adenosine 5'-triphosphate. ATP's use in the synthesis of biomolecules require enzymes. Before their emergence, alternative energy sources, perhaps assisted by simple catalysts, are believed to have mediated the activation of carboxylates and phosphates for condensation reactions. The authors showed that the chemical energy inherent in isonitriles can be harnessed to activate nucleoside phosphates and carboxylic acids through catalysis by acid and 4,5-dicyanoimidazole under mild aqueous conditions. Simultaneous activation of carboxylates and phosphates provides multiple pathways for the generation of reactive intermediates, including mixed carboxylic acid-phosphoric acid anhydrides, for the synthesis of peptidyl-RNAs, peptides, RNA oligomers and primordial phospholipids.

In 2022, Bromberg et al [45] did computational exploration of similarities among metal-binding protein structural motifs. Using a novel, structure-guided sequence analysis of proteins, the authors explored the patterns of evolution of enzymes responsible for these reactions. They found that the folds that bind transition metal–containing ligands have similar structural geometry and amino acid sequences across the full diversity of proteins. Similarity across folds suggested the availability of key transition metals over geological time. The authors believe that transition metal–ligand binding had a small number of common peptide origins. The structures central to their similarity network came primarily from oxidoreductases, suggesting that ancestral peptides may have also facilitated electron transfer reactions.

Fontecilla-Camps [46] argues that although nickel (Ni) is a minor element of the Earth's crust, it played a major role in the evolution of life. This metal is a component of the active sites of several archaeal and bacterial anaerobic enzymes essential for bioenergy processes such as H2 and CO oxidation and CO2 fixation. Furthermore, Ni of meteoritic origin was probably involved in primordial organic phosphorylations. However, Fontecilla-Camps points out that depending on its concentration, Ni can also be extremely toxic to most species.

In 2023, Subbotin and Fiksel [47] measured UV absorption in aquatic solutions of several ferrous mineral salts assumed to be present in Archean pools. These direct measurements of UV light absorption supplemented and reinforced the hypothesis that the UV-shielding ability of the Archean waters could have protected submerged liposomes from damaging solar UV radiation.

Papineau et al [48] proposed that Chemically Oscillating Reactions (COR) played a role during the prebiotic cycling of carboxylic acids, furthering the new model for geology where COR can also

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explain the patterns of diagenetic spheroids in sediments. CORs are abiotic, spontaneous, out-of-equilibrium, redox reactions that involve the decarboxylation of carboxylic acids with strong oxidants and strong acids to produce CO_2 and characteristic self-similar patterns.

Ernst et al [49] studied routes towards abiotic methane and ethane formation under early-earth conditions from methylated sulfur and nitrogen compounds with prebiotic origin. The authors feel that this may have shaped the chemical evolution of the atmosphere prior to the origin of life and beyond.

Bada [50] hypothesizes that lightning associated with volcanic island eruptions created focal points for the generation of prebiotic ingredients and ultimately the origin of life. He feels the early Hadean eon (>4Ga) may have had a periodically ice-covered global ocean and limited subaerial landmass which could have resulted in infrequent lightning occurrence.

2.2. Organic Composomes and Various Organic Metabolism-First Models

Doron Lancet and Daniel Segre at The Weizmann Institute of Science in Israel in 1998 originated the idea of Graded Auto-Catalytic Replication Domains (GARD) [51]. Segre and Lancet provided a rigorous kinetic analysis of simple chemical sets that manifest mutual catalysis. Catalytic closure was hypothesized to sustain self-replication up to a critical dilution rate related to the graded extent of mutual catalysis. The authors explored the behavior of vesicles containing GARD "species." Mutual catalysis was seen to be governed by a statistical distribution. Some GARD vesicles displayed a significantly higher replication efficiency than others. Thus, GARD was viewed as a simple model for primordial chemical selection of mutually catalytic sets.

Work along the lines of GARD has continued through the years [52–60]. Recently, Lancet and A.M. Segre have revisited some of the original theoretical models dealing with the chemical emergence of "life-like" properties in prebiotic systems. Special emphasis was given to models involving random assemblies of mutually catalytic organic molecules, as opposed to scenarios in which individual molecular species are endowed with the capacity of self-replication. They believe that some of these metabolic reactions were initially catalyzed by less sophisticated and less specific catalysts, such as small organic molecules, metal ions, minerals, short RNA polymers, prebiotic amino acids or peptides. Smaller molecules could have persisted throughout evolution, gradually becoming incorporated into protein enzymes as catalytic cores or cofactors. They envision geochemically available prebiotic catalysts like transition metals, iron-sulfur clusters and organic cofactors.

Lipid world micelles and vesicles play a considerable role in compartmentalization of these mutually catalytic sets of simple organic molecules. They are seen to have undergone selection, evolution, and transfer of chemical information.

Segre, Lancet and Shenhav's mutually catalytic assemblies, devoid of sequence-based biopolymers, are also envisioned to entail a primitive information transfer system, exclusively based on idiosyncratic chemical compositions. This is imagined as the inheritance of spontaneous "compositional genomes." Of course, their definition of "information" is very different from Szostak's "functional information" [61–66] or Abel's more refined "Prescriptive Information (PI)" [67–69].

A continuous flow of nutrients into and out of reaction vessels has shown that simple mixtures of thiols and thioesters could display a wide range of dynamical properties, such as biostability, oscillations and autocatalysis [70,71]. These authors believe that collectively autocatalytic cycles and biological networks could have emerged from simple mixtures of prebiotically plausible chemicals and mineral surfaces held out of equilibrium.

The new synergistic discipline of Chemobrionics [25] addresses self-ordering precipitation processes, such as chemical gardens forming biomimetic micro- and nanotubular forms. Nonequilibrium physicochemical systems are studied. The assembly of material architectures under a flux of ions is exploited in various applications. Chemobrionics requires a combination of expertise in physics, chemistry, mathematical modeling, biology, and nanoengineering, complex theory, and

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nonlinear and materials sciences [25]. Unclear is where all this expertise came from in a prebiotic environment.

Many others in the last decade have contributed to various chemical evolution and molecular evolution models [72–78].

In 2022, Heylighen, Beigi and Busseniers [79] characterize living systems as resilient "chemical organizations", i.e. self-maintaining networks of reactions that are able to resist a wide range of perturbations. They acknowledge that dissipative structures, such as flames or convection cells, are also self-maintaining, but much less resilient. They fail to distinguish between physico-dynamic self-ordering and the imaginary self-organization of abstract formalisms, however [80]. They try to understand how life could have originated from “self-organized” structures, and evolved further by acquiring various mechanisms to increase resilience. Negative feedback, buffering of resources, and degeneracy (producing the same resources via different pathways) are all considered. Catalysis, enzymes, regulation by "memory" molecules such as DNA, etc are all discussed without resolution.

Vanchurin et al [81] outline a phenomenological theory of evolution and origin of life by combining the formalism of classical thermodynamics with a statistical description of learning. They feel that the maximum entropy principle was somehow constrained by the requirement for minimization of the loss function. It is not at all clear to this author (Abel) how an inanimate environment would or could constrain minimization of loss of function when a prebiotic environment possesses no sense of “function,” no valuation of its importance, and no effort to pursue utility of any kind.

Zlenko, Zanin, and Stovbun [82] point to the ability of molecules to form highly elongated helical supramolecular structures (strings) when precipitating from homochiral solutions. The strings forming can be accompanied by spontaneous splitting and/or chiral purification of the initially racemic mixture. The authors describe the strings forming in homochiral amino acid solutions. The source of this initial homochiral amino acid prebiotic solution in this paper was not explained.

Qu et al [83] pursue a photothermal (PT) effect-derived enantioselective desorption strategy based on homochiral Au/TiO2 nanotubes (NTs). Using 3,4-dihydroxyphenylalanine (DOPA) as the model enantiomer, they achieved an obvious selective desorption of L/D-DOPA by NIR light-triggered local temperature enhancement. Molecular docking simulation further verified that the distinct affinity precipitated by the different hydrogen bonds between homochiral sorbent and target enantiomers was the origin of enantioselective desorption. This desorption strategy provided an alternative approach for the selective separation of chiral molecules.

Dong et al [84] pursue advancements on the question of the derivation of homochirality in abiogenesis. The exfoliation of molecular crystals, in which repeat units are held together by weak non-covalent bonding, could generate a wide range of two-dimensional crystalline materials with diverse surfaces and structural features. In this respect, they represent a distinct type of material in which molecular components are all equally exposed to their environment, as if in solution, yet with properties arising from cooperation between molecules, because of crystallinity. This unusual nature is reflected in the molecular recognition properties of the materials, which bind carbohydrates with strongly enhanced enantiodiscrimination relative to individual molecules or bulk three-dimensional crystals.

Kuhne et al [85] show that the statistical prevalence of homochiral crystallization of [Mn(4-OMe-Sal2323)](+) in five lattices with different achiral counterions suggests that the chirality may be directed by the 4-OMe-Sal2323 ligand.

Lin et al [86] point out that the prebiotic availability of 5'-nucleoside triphosphates (NTPs) has been unresolved and other derivatives of nucleoside-monophosphates (NMPs) have been studied as a result. However, the latter approach necessitates a change in chemistries when transitioning to biology. Their one-pot protocol for simultaneous generation of NaPs and NTPs suggests that the transition from prebiotic-phosphorylation and oligomerization to an enzymatic processive-polymerization can be more continuous than was previously thought.

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Kondratyeva et al [87] point out that no ribozymes with processive polymerase activity have been yet discovered or synthesized. They also discuss the mutual need of proteins and nucleic acids for each other in the current world. Thus, many authors propose the early evolution scenarios based on the co-evolution of these two classes of organic molecules (originating mainly from the work of Wong discussed below).

Fiore et al [88] argue that "prebiotic lipidomics" should play a major role in abiogenesis research. Unique hydrophobic phospholipids are essential components of biological membranes and are involved in cell signalization, in several enzymatic reactions, and in energy metabolism. The monolayers possess complex chiral surfaces derived partly from individual supramolecular coordination complex components but also from interactions with neighbors.

Deamer, Cary and Damer [89] coin the word “urability” to distinguish habitability on exoplanets from also having the environment allowing potential of abiogenesis. They address the geochemical factors that support plausible localized zones that are conducive to the chemical reactions and molecular assembly processes that would have been required for the origin of life. Thus, “urable worlds” are seen as planetary bodies that can sustain both abiogenesis and ongoing survival of life on other bodies in our own solar system and exoplanets beyond.

Castelvecchi [90] demonstrated that RNA molecules can link short chains of amino acids together. The author hypothesizes that protocells could have arisen from an RNA-protein mix. All of the problems remain, however, of how a functional sequence of levo-only amino acids (primary structure) could have been selected and engineered that would have anticipated secondary structure and tertiary fold requirements needed for any one of scores of required homochiral enzymes to form all at the same time and place.

de la Pena and Gago-Zachert [91] find that small circular genomes can encode self-cleaving RNA motifs or ribozymes. They suspect that autocatalytic ribozymes not only provided a common rolling-circle replication mechanism for subviral viroids and viral satellites of circular RNAs (circRNAs), but also a tentative link with the origin of life as molecular fossils of the so-called RNA world.

Bose et al [92] believe that the protoribosome, which is still embedded in the contemporary ribosome, is a vestige of the primordial ribosome. The authors showed using the "fragment reaction" and its analyses by MALDI-TOF and LC-MS mass spectrometry techniques, that several protoribosome constructs are indeed capable of mediating peptide-bond formation. The authors believe that the protoribosome may be the missing link between the RNA dominated world and the contemporary nucleic acids/proteins life.

In 2023, Tagami and Li [93] found that peptides with both hydrophobic and cationic moieties (e.g., KKVVVVVV) form beta-amyloid aggregates that adsorb RNA and enhance RNA synthesis by an artificial RNA polymerase ribozyme. In addition, they found that a simple peptide with only seven amino acid types (especially rich in valine and lysine) can fold into the ancient beta-barrel conserved in various enzymes, including the core of cellular RNA polymerases.

Meyer et al [94] performed Next-Generation Sequencing (NGS) experiments in membraneless compartments called complex coacervates. They characterized the fold of many different transfer RNAs (tRNAs) simultaneously under the potentially denaturing conditions of these compartments. They found that natural modifications favor the native fold of tRNAs in these compartments, suggesting that covalent RNA modifications could have played a role in proto-metabolism.

Babajanhan et al [95] distinguish between reproducers and replicators. Reproducers are cells and organelles that reproduce via various forms of division and maintain the physical continuity of compartments and their content. Replicators are genetic elements (GE), including genomes of cellular organisms and various autonomous elements, that both cooperate with reproducers and rely on the latter for replication. All known cells and organisms comprise a union between replicators and reproducers. The authors explore models in which cells emerged via symbiosis between primordial "metabolic" reproducers (protocells) and mutualist replicators. They believe that “metabolic reproducers evolved, on short time scales, via a primitive form of selection and random drift.

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Fields and Levin [96] feel that free energy and thermodynamic requirements on bidirectional "information" exchange between a system and its environment can generate complexity. Because they fail to distinguish between mere "complexity" vs "conceptual complexity," they feel that free energy and thermodynamics leads to the emergence of hierarchical formal computational architectures and biosystems that operate far from thermal equilibrium. They believe the environment of some natural systems increases its ability to predict system behavior by "engineering" the system towards increased morphological complexity, and hence larger-scale, more macroscopic behaviors. Such "regulative development" is thought to become an environmentally-driven process in which "parts" are assembled to produce biosystems with predictable behavior.

3. RNA/Ribozyme World and RNA/Peptide World Models

RNA world models emerged as the great new hope decades ago [97]. They remained popular for many years. RNA provided hope of simultaneous information retention, heritability and catalysis. But the prebiotic formation of even just ribose was quickly recognized to be problematic, let alone the spontaneous formation of full-length catalytic RNA's. The instability of RNA posed further extreme limitations. Longer RNA catalytic properties also did not live up to widely promoted promises.

Bernhardt [98] acknowledged the many problems associated with the RNA world hypothesis, but felt encouraged by the discovery of some extremely small ribozymes, possible first tRNA's, the benefit of an acidic pH environment, nonribosomal peptide synthesis, and a possible replicase origin of the ribosome. Bernhardt pointed out that non-coding RNAs such as ribosomal RNA and tRNA were able to replicate prior to the evolution of ribosomal protein synthesis.

Nevertheless, an RNA analog theory soon displaced RNA World theory mostly for biochemical reasons. But RNA analogs retained many of the RMA World explanatory problems. A rarely emphasized one is that the likelihood of a stochastic ensemble of ribonucleosides prescribing that RNA's auto-catalysis was extremely unlikely. Far worse was the utter implausibility of that exact same auto-catalytic sequence simultaneously prescribing any significant protometabolic function. Such miraculous duality of prebiotic stochastic ensemble functionality would be statistically prohibitive.

Another major problem was elucidating how any prebiotic RNA template could have itself acquired any Prescriptive Information (PI) in its primary structure that would be worth copying and propagating. But an inanimate environment has no sense of value or function to start with. No goal of utility exists. The conundrum of pragmatic directionality remains the most serious problem in every abiogenic model. No such impetus as "in order to . . . " exists in molecular evolution. Yet this is by far the number one "explanatory mechanism" provided in molecular evolution papers.

Saito in 2022 [99] sees DNA having formed from RNA. Thus, abiogenesis would have to trace back to either an RNA World or a Protein World model. He views the spontaneous formation of both proteins and RNA extremely unlikely. He argues that in RNA viruses, RNA serves as both genome and catalyst of peptide formation. But, of course, this presupposes "genome" rather than explaining its origin. He acknowledges that 1) RNA is too complex a molecule to have arisen prebiotically; 2) RNA is inherently unstable; (3) catalysis is a relatively rare property of long RNA's (4) the catalytic repertoire of RNA is very limited.

Forster in 2022 [100] attributes what he considers to be the death of the RNA World to RNA's polyanionic charges that could not be covalently neutralized stably by phosphotriester formation. These charges prevented development of hydrophobic cores essential for integration into membranes and many enzymatic reactions. Thus, any ribo-organisms, even if formed, would have quickly become extinct. It should also be pointed out that no one has achieved bona fide self-replication of RNA, especially in a prebiotic environment.

Forster also argues that the phosphotriester modification of DNA is far more stable. Forster points to the catalytic superiority of the 20 amino acid side chains and hypothesizes that catalytic

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polypeptides formed prior to DNA. Thus, more recent models have incorporated the addition of peptide roles in conjunction with RNA.

Müller et al in 2022 [101] suggest that non-canonical vestige nucleosides in RNA found today in transfer and ribosomal RNAs are able to establish peptide synthesis directly on RNA. The formed RNA–peptide chimeras are comparatively stable. The authors thought that it was conceivable that some of these structures learned, at some point, to activate amino acids by adenylation and to transfer them onto the ribose OH groups to capture the reactivity in structures that were large and hydrophobic enough to exclude water. Exactly how such molecules would have *learned* anything is not clear. Müller et al suggest that this would have been the transition from the non-canonical nucleoside-based RNA–peptide world to the ribosome-centered translation. Thus, an RNA/Peptide World seemed to offer more promise than the original RNA World.

4. Co-Evolution Models Attempting to Link Biochemistry with the Translation Needed for Replication of Nucleic acids

Another major thrust of abiogenic research right up to the present has been in the proposal of a link between biochemistry, translation and eventual code origin. The reality of multiple kinds of coding in all known life forms is impossible to deny. Neither coding nor biosemiosis can be reduced to mere metaphor [102,103] .

J.T. Wong first published his co-evolution model nearly 50 years ago [104,105]. Wong proposed that the structure of the genetic code was determined by the sequence of evolutionary emergence of new amino acids within the primordial biochemical system. His work on the model has continued through the decades [106–111].

The prediction of the coevolution theory of the genetic code that the code should be a mutable code has led to the isolation of optional and mandatory synthetic life forms with altered protein alphabets [106].

Co-evolution models postulate that the emergence of translation was necessary for the replication of nucleic acids. This is in contrast to the RNA world hypothesis which believes the emergence of translation was preceded by the era of self-replicating RNAs. Co-evolution scenarios require the highly unlikely simultaneous emergence of two classes of organic molecules, as well as the emergence of synchronized replication and translation. But the authors point out the major advantage of co-evolution models is that they explain the development of processive and much more accurate protein-dependent replication.

It is debatable, however, whether the "explanation" of the emergence of synchronized replication and translation is actually provided. An attempt is made admirably to link the two. But the means in the final analysis is more like magic than a scientific explanation of "how." The co-evolution model presupposes not only evolutionary emergence of amino acids, but in a certain efficacious sequence. First, what was the basis for *the selection needed* in molecular evolution in an abiotic environment? And what is the basis for presupposing the existence of any biochemical "system"? Non-equilibrium phase transitions are not formally controlled systems. Mere uncertainties do not program or compute. They are not true "systems." We just call them systems, like we carelessly do with merely self-ordered weather fronts. Then we compound the folly by calling such self-ordered dissipative structures "self-organized" rather than the self-ordering they actually manifest [80].

Wong believes that genetic information arose from replicator induction by metabolite in accordance with the metabolic expansion law. Messenger RNA and transfer RNA stemmed from a template for binding the aminoacyl-RNA synthetase ribozymes employed to synthesize peptide prosthetic groups on RNAs in the Peptidated RNA World. Coevolution of the genetic code with amino acid biosynthesis is believed to have generated tRNA paralogs that identify a last universal common ancestor (LUCA) of extant life close to *Methanopyrus*. This might suggest that archaeal tRNA introns were the most primitive introns. The anticodon usage of *Methanopyrus* might have been an ancient mode of wobble.

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Wong generated the co-evolution model of molecular evolution in hopes of sidestepping any need for active selection [106,111]. But the limits of physicodynamics is considerable when trying to explain abiogenic molecular biological instruction, control, transcription and translation. Selection of amino acids and nucleosides for sequential rigid polymerization precedes any realized utility. Nothing is alive yet to differentially survive. Prebiotic selections could not have been passive or secondary [112,113].

Barricelli [114] considered whether groups of tRNAs might have attracted certain amino acids. He envisioned a complementarity between each tRNA and the genome segment from which it was originally copied. He called this the "pairing release hypothesis."

Rodin and Rodin [115] observed that if the table of the genetic code is rearranged to put complementary codons face-to-face, the code displays latent mirror symmetry with respect to two sterically different modes of tRNA recognition. These modes involve distinct classes of aminoacyl-tRNA synthetases (aaRS's I and II) with recognition from the minor or major groove sides of the acceptor stem, respectively. Perhaps a potential selective advantage arose from the partitioning of aaRS's into two classes, where the two aaRS classes were originally encoded by the complementary strands of the same primordial gene.

Massimo DiGiulio has since led the way in biological code origin studies, with his most recent paper analyzing the origin of the genetic code in the light of the evolution of biological catalysts. He hypothesizes rudimentary forms that the genetic code may have assumed. He believes catalysis may have been performed by ions or by low molecular weight molecules, such as nucleotide coenzymes [116].

Romeu Guimaraes since the early 1990's has been refining his Self-Referential Genetic Code [117,118]. Guimaraes sees the genetic code as the correspondence between 'letter' units that cells utilize for translation: triplets of bases in the producers (genes) and amino acids in the products (proteins). The self-referential model indicates that the codes resulted from proto-tRNA dimer-directed protein synthesis. The dimerized proto-tRNAs became codes when the peptides they produced bound back to them and stabilized the correspondence between the units and the protein production system. Anticodons are seen as representative sites of the initial binding oligomers that guided the complementariness at dimerization.

In 2023 Prosdocimi and de Farias attempted to explain everything [119]: how nucleotides and other biomolecules could be made prebiotically in specific prebiotic refuges; (ii) how the first molecules of RNAs were formed; (iii) how the proto-peptidyl transferase center was built by the concatenation of proto-tRNAs; (iv) how the ribosome and the genetic code could be structured; (v) how progenotes could live and reproduce as "naked" ribonucleoprotein molecules; (vi) how peptides started to bind molecules in the prebiotic soup allowing biochemical pathways to evolve from those bindings; (vii) how genomes got bigger by the symbiotic relationship of progenotes and lateral transference of genetic material; (viii) how the progenote LUCA has been formed by assembling most biochemical routes; (ix) how the first virion capsids probably emerged and evolved; (x) how phospholipid membranes emerged probably twice by the evolution of lipid-binding proteins; (xi) how DNA synthesis have been formed in parallel in Bacteria and Archaea; and, finally, (xii) how DNA-based cells of Bacteria and Archaea have been constituted.

Missing from co-evolution models is pragmatic directionality and the steering and control needed to orchestrate a more holistic formal scheme. Although both Digiulio and Guimaraes deal with "coding," their approach seeks to reduce "code" to mere physicodynamics. The essence of formal representationalism using symbolically-coded instructions and message meaning is lost.

4.1. Biosemiotics and Code Biology

Biosemiosis interest began with Charles Peirce [120]. Many have contributed to the development of this field, with their most recent contributions including von Uexküll [121], Varela FJ [122,123], Pattee [124–127], Rosen [128–131], Sebeok [132,133], Hoffmeyer [134–136], Emmeche [137] Kull [138–140], El-Hani [141], Sharov [142–145], Barbieri (cited below), and Kull [138,140,146–149]

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Rosen's contribution was considerable [128–131]. Most significant was his dichotomy between formal vs physico-dynamic. But from an abiogenist's perspective, his notion of selection was unfortunately limited to the very inadequate after-the-fact, secondary, passive selection of "natural selection." The orchestration of even a protometabolism required active, not passive selection. It required selection "in order to . . . ," not merely selection "from among" already optimized functions or organisms.

Marcello Barbieri has extensively emphasized the role of a myriad of codes in biology [150–155].

The Biosemiosis and Code Biology movements in general [150,152,156–160] stress the reality of life's subcellular *messaging*. Little is found in biosemiotic literature, however, addressing the need for *prescription* of message content (instructions) at the prebiotic subcellular level [161]. Not even a protometabolism just happens. Biochemical pathways have to lead or pushed somewhere useful. Sophisticated bifunctionality and biosystems have to be integrated into connected pathway circuits with configurable switch settings. This orchestration had to have arisen in an inanimate pre-evolutionary environment prior to LUCA (Last Universal Common Ancestor). Feedback mechanisms depend upon already- existing biochemical pathways.

Barbieri in the last decade has pursued a much more materialistic approach to biological codes than most other biosemioticians. Probably fearing the accusation that biosemiotics emphasis would be accused of not being scientific, Barbieri shifted his emphasis to "Code Biology." He apparently felt this would de-emphasize the essential *meaning* aspects of message content. But the reality of biomessage meaning is unavoidably inherent in programming and other forms of biological instruction and control. Life is computation. Computation is formal, not physical.

Many others have pursued the biosemiotic realities of subcellular life. [69,159–170].

Most biosemioticians are more willing than Barbieri to acknowledge the need for "interpretation" of message meaning. Almost none admit to the need for active selection of signs/symbols. For the most part, biosemioticians still remain locked into a primarily materialistic axiom that precludes investigating the required active selection of prebiotic sign and rule selection. Physicodynamics offers no hint of explanation for any message meaning or purpose, including the co-evolution models of "code" discussed above.

The reality of biological code, message and the prescription of function at distant locations within the cell is quite real rather than metaphorical [69,103,113,171–177].

Federico Vega [178] and Kravchenko [179] disagree with Barbieri that interpretation is irrelevant for biosemiosis. According to Vega and Kravchenko, Barbieri views coding as the sole mechanism of semiosis in the organic world. These two authors, on the other hand, argue that the concept of "code" as a one-to-one correspondence between two sets of objects (sign vehicles) cannot explain living organization. Interpretation is seen as an organism's adaptive response to the environment. Vega and Kravchenko both advocate adopting a more comprehensive biosemiotic theory that focuses on its relational nature. They emphasize the role of selection of interpretable signs to create instruction [178].

Semiosis is mediated through the representationalism of signs/symbols and arbitrarily agreed-upon rules by sender and recipient. This is exampled by not only the codon table, but also by superimposed translational pausing coding [180]. Interpretation of symbols and symbol meaning is *everything* if any sophisticated biofunction is to be realized. A physicodynamic cause and effect linkage is not what mediates biosemiosis. Meaning constitutes the essence of the message. But we are not just dealing with biological subcellular messaging. Abiogenists must address *prescription* of formal biofunction and biosystems.

Kull wisely ponders a non-anthropomorphic biological understanding of free choice in a very recent paper [146]. All too many biosemiosis papers center only on organismic sentience, perception, agency and psychology. They are therefore of little interest to abiogenists. Organisms do not yet exist. Despite this shortcoming, Kull's interest in non-anthropocentric biological choice is most welcome.

Kull analyses the structure and roles of biological choice [146]. He rightly argues that choice does not necessarily require purpose. But one wonders what choice without intent would accomplish. Of

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interest to abiogenists is the orchestration of protometabolism in an inanimate environment. Organismal sentience and choice are not relevant yet. Subcellular, efficacious, active selection at many different levels had to take place prior to the realization of the many needed productive pathways and integrated circuits of any protolife. Life is undeniably programmed and cybernetically processed. How was Turing's "halting problem" overcome? Certainly not by choice without purpose. Coin flip "choices" at mere bifurcation points are not decision nodes. Coin flips do not constitute efficacious programming. Neither do "non-equilibrium phase transition instabilities" championed by Wills and Carter [181–184]

Alexei Sharov has also addressed many semiotic and proto-cybernetic issues [142–145]. Sharov and Vehkavaara wisely distinguish between protobiosemiosis vs. eubiosemiosis [145]. They contend that protobiosemiosis started with signs associated with actions within the origin of life. How would "associated with" translate to "causation" of any biosystem? They feel that eubiosemiosis started when evolving agents acquired the ability to track and classify objects. Do micelles and coacervates "track and classify?" They speak of "proto-signs" that can be classified into proto-icons. Proto-icons can signal via single specific interaction. Proto-indexes can combine several functions. Proto-symbols are processed by a universal subagent equipped with a set of heritable adapters [145]. This is all very imaginative. How scientifically plausible these successively linked steps are remains problematic.

Peter Wills is among many who have attempted to find a materialistic basis for coding and semiosis [184]. In his and Carter's most recent papers [181–187], we find somewhat beclouded models of origin that make falsification difficult. Empirical support for mere instabilities in non-equilibrium phase transitions writing Prescriptive Information and controls needed to orchestrate even a protometabolism remains painfully lacking.

Biosemioticians, in particular, frequently attempt to perform delicate materialistic dances around formal representational concepts such as "signs," 'symbols," "tokens, "meaning," arbitrary "rules" (as opposed to laws), and "agency" in the selection of symbols that alone makes semiosis possible. Although tokens may be "physical symbol vehicles," they still must be chosen to generate any "meaning" in any material symbol system (MSS) [188,189].

Rocha has continued to address codes and semiosis in greater detail [190–194], although the semiotic processes supposedly elucidated in all of these models often resembles more of a shell game and linguistic obfuscation than anything scientific.

Kalevi Kull is perhaps the bravest of all biosemioticians in acknowledging the third fundamental category of reality [172], Choice Causation, and its necessity for any form of semiosis, biosemiosis included. But Kull does not seem to address the necessity of choice causation at the sub-cellular, pre-biotic, molecular biological level of abiogenesis. It is not sufficient to discuss organisms' choices. Choice Causation is necessary to orchestrate any sub-cellular protometabolism or initial protocell.

In 2022, Chatterjee and Yadav [195] defined three different prebiotic information systems: analog, hybrid, and digital. They hypothesized that the Analog Information System (AIS) was manifest early in abiogenesis, was expressed in chiral selection, nucleotide formation, self-assembly, polymerization, encapsulation of polymers, and division of protocells. AIS created noncoding RNAs by polymerizing nucleotides that gave rise to the Hybrid Information System (HIS). In their scenario, the HIS employed different species of noncoding RNAs, such as ribozymes, pre-tRNA and tRNA, ribosomes, and functional enzymes, including bridge peptides, pre-aaRS, and aaRS (aminoacyl-tRNA synthetase). Some of these hybrid components supposedly built the translation machinery step-by-step. Note the hidden presupposition of "goal" in "step-by-step." The HIS ushered in the Digital Information System (DIS), where tRNA molecules become molecular architects for designing mRNAs step-by-step, employing their two distinct genetic codes. They then compared the three kinds of biological information systems with similar types of human-made computer systems. The authors seemed oblivious to the full import of their own analogous comparison with human cybernetics. They could not see the obvious steering and control mechanisms that would have been required for bona fide organization and orchestration of any one biosystem they described. No explanation is provided for how mere physico-chemical causation could have accomplished any one of these

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biological computational haltings. No perception was apparent of the need for Prescriptive Information (PI) [68,69] (instructions and recipe), and the processing of that programming required for the engineering and progression of the integrated circuits that they describe.

Marijuán and Navarro [196] focus on biomolecular information flow and processing in the development of biological complexity. They incorporate fundamental conceptualizations on the mechanisms of molecular recognition and informational architectures, the life cycle, and the characterization of meaning. Their informational approach depicts an indefinite series of recursion processes performed in the open-ended environment of the real world, potentially affected by multiple contingencies modifying the informational architectures involved in recursion. They pursue a power law that might interconnect the variability outcomes of the different evolutionary "vehicles" or variation modes.

Prinz [197] believes that biocomplexity can be attributed to the presence of codes, a unique organizational principle of living systems. He distinguishes biocomplexity from mere complexity, and attempts to measure "biocomplexity." Prinz uses a code-based measure of biocomplexity expressed as a simple formula: "codes/components = complexity." He feels this allows quantification of biological complexity across a wide range of biosystems and biological taxa. His method combines informational concepts (i.e., Marcello Barbieri's conception of biological codes [152]) with quantitative behavior of biomolecular components. The proposed formula incorporates both the number of components in a cell and the number of interactions constituting its underlying molecular networks. All molecular processes in between anabolism and catabolism require tight regulation and coordination. The control of these processes helps define Prinz's definition of "biocomplexity."

In 2023, Prinz [156] draws further attention to the potential contribution of biological codes to the course and dynamics of evolution. Prinz argues that the concept of organic codes, developed by Marcello Barbieri [150–154], has fundamentally changed our view of how living systems function. The notion that molecular interactions built on adaptors that arbitrarily link molecules from different "worlds" in an arbitrary/conventional, rule-based way, departs significantly from the law-based constraints imposed on living things by physical and chemical mechanisms. Prinz argues that living things operate on the basis of rules, whereas non-living things behave according to laws. He argues that this critical distinction is rarely if ever considered in current evolutionary theory. The many known codes allow quantification of codes that relate to a cell, or comparisons between different biological systems and may pave the way to a quantitative and empirical research agenda in code biology. Prinz advocates a simple dichotomous classification of structural and regulatory codes. This classification can be used as a tool to analyze and quantify key organizing principles of the living world, such as modularity, hierarchy, and robustness, based on organic codes. The implications for evolutionary research are related to the unique dynamics of codes, or "Eigendynamics" (self-momentum) and how they determine the behavior of biological systems from inside, whereas physical constraints are imposed mainly from outside. A speculation on the drivers of macroevolution in light of codes is followed by the conclusion that a meaningful and comprehensive understanding of evolution depends on including codes into the equation of life.

Douglas Axe [198] in a quality paper in the Journal of Molecular Biology calculated the improbability of functional protein folds to be only one in 10^77. Tertiary protein structure doesn't just happen. We have not even begun to address biochemical pathways or biosystems here. We are just talking about achieving the functional folding of a single protein! How was this statistical prohibitiveness narrowed down in a prebiotic environment to achieve the selection of what was needed? Did an inanimate environment sense or care about "needs"?

4.2. Remaining Hurtles in Abiogenesis Research

Superb synthetic chemist, materials scientist and nanotechnologist Professor James Tour at Rice University enumerates many of the challenges that still face abiogenic research in his 14-lecture series on abiogenesis [199]:

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- Critical sequencing of reactions: an inanimate environment has no sense of *sequencing* reactions needed for synthesis. Any organic chemist knows that the correct order of addition of each reagent is absolutely essential to have any hope of producing a purified adequate "yield."
- The slightest impurities ruin synthetic organic chemistry.
- Prebiotic environments cannot purify reactants, or achieve their delicate quantities needed for synthetic chemistry.
- Instead of using sequentially produced in-lab reagents in successive steps, extrinsically supplied homochiral populations of moieties must be ordered and used from Sigma-Adrich-like chemical plants. To produce a pure moiety, the engineered products themselves require homochiral seeding. No such seeding or processes were available in prebiotic environments.
- Spontaneous reactions cannot synthesize significant quantities of useable product. Useless tars result instead.
- Carbon forms strong bonds that do not hydrolyze easily, but can remodel with enzymes. Where did the highly specific functional enzymes come from in an inanimate environment?
- Hypothesized Silicon Life chemically dead-ends. The bonds are too rigid.
- Purely physicalistic abiogenic reactions in plausible prebiotic environments don't know how or when to stop.
- Highly intelligent chemists must keep separating out from ongoing reactions what is wanted and needed to prevent the inevitable tar end-product.
- It is very difficult to undo unhelpful reactions. Reactions cannot back up and do retakes with different moieties.
- Molecules form innumerable unwanted cross-reactions.
- "Helpful" molecules degrade almost as fast as they form. The half-life of Ribose is only five hours. All ribose would have been gone, even if it had formed, within two days in a magnesium rich early earth crust.
- Eschenmoser spent a lifetime trying to make functional RNA. He couldn't even produce five-carbon-sugar ribose naturalistically.
- The yield is often only 1-2% of most organic syntheses, creating a mass transfer crisis. This problem arises with any net movement of mass from one location or phase to another. Mass Transfer is involved in evaporation, drying, precipitation, absorption, membrane filtration, distillation, etc. With such low yields, even in carefully controlled synthetic chemistry labs, any environment soon runs out of resources.
- Aqueous environments prevent dehydration synthesis.
- Polypeptides cannot form in the presence of sugars or aldehydes.
- Amino acids and sugars cross react, resulting in insoluble polymers.
- Molecules oxidize. Ammonia in a reducing environment is anything but helpful. A reducing environment is even more degrading. As of 2011, papers in such journals as Nature began presenting evidence and concluded that early earth's atmosphere was NOT reducing [200]. It does not really matter, however, whether it was a reducing or oxidizing environment. The necessary chemistry would not have spontaneously proceeded in either environment.
- Amino acid mixes are not just of the 20 classic needed amino acids. Many other poisonous amino acids are mixed in that would have jammed abiogenesis.
- Four fundamental kinds of molecules are needed for abiogenesis, not just proteins. Lipids, polysaccharides and nucleotides are also essential. All of these players present tremendous engineering problems to produce. Even then, they are only racemic.
- The possible permutations of polysaccharides and lipids alone that can form is mind-boggling. Abiogenesis is not just a protein or nucleoside-formation problem. Selection of only the correct moieties is statistically prohibitive. Every published model of abiogenesis thus far can be shown to measure out with a Universal Plausibility Metric of ξ equaling <1.0. This requires peer-review rejection of that model and manuscript for reason of scientific implausibility (The Universal Plausibility Principle) [201–203].
- How many ways can 60 D-glucoses be linked together to make Starch? Just six repeated units of D-glucose can form one trillion different branching and stereochemically distinct hexa-

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saccharides. Novice abiogenists don't appreciate the number of permutations from which the correct one must be isolated and used.

- Nobody has ever made a self-purifying starch necessary for life in a relatively useful stereochemical form in a prebiotic-like environment. This doesn't even address a purely homochiral right-handed-only ribose. Prebiotically plausible ribose generation models are all racemic and in such a mixture one could never find R-ribose exclusively.
- Carbohydrate polymerization is statistically prohibitive without highly specific enzymes that were simply not present in a pre-biotic environment.
- Polysaccharides have vast numbers of carbohydrate appendages. They have highly unique assemblies and important functional three-dimensional structures, the same as proteins. Polysaccharides (carbohydrates), therefore, contain enormous opportunity for information retention, which life fully uses.
- Even when one already has D-glucose, it can have a large number of other possible forms mixed in as pollutants that terminate any hope of abiogenesis.
- 5-Carbon Carbohydrate is the hardest component of life to explain. Eshenmoser spent most of his career trying to make 5-carbon ribose so that he could start to make RNA. All he could make was 6-membered sugars rather than the five-membered sugars. So he tried to make an analog of ribose. He failed in the 70's and early 80's. Synthetic chemists have done better since, but only by literal chemical engineering, not by "natural process," and especially not by prebiotic natural process.
- DNA tripartite needs ribose. Ribose is only one of the building blocks of the building blocks!
- Virtually none of the building block precursors form spontaneously, especially not with enantiomeric excess. Homochirality of sugars and amino acids needs to be 100% for electron spin up or down to make life work.
- Only two of the twenty amino acids can crystalize spontaneously to get only the L-optical isomer. Artificially manufactured L-amino acids are needed to crystalize additional L-amino acids. But even then, the yield is only around 1-2%. A 100% homochiral yield is needed.
- Prebiotic reactions had no control over critically-needed stereochemistry.
- Sophisticated enzymes not only make reactions possible, but speed them up by many orders of magnitude. Abiogenesis could never have occurred at the ridiculously slow pace of reactions apart from sophisticated enzymes. Early enzyme-like moieties would have been totally inadequate.
- Enzymes check things out to make sure the reaction sequence is what is needed. Thus reaction rate does not constitute the only need for enzymes.
- But enzymes, along with the other three essential classes of molecules needed for abiogenesis, cannot be made themselves without other enzymes, and without nucleosides.
- Enzymes are even needed for polysaccharide and proper active transport lipids.
- Dehydration synthesis of peptides and proteins cannot occur in an aqueous environment without very creatively designed and engineered enzymes.
- All components must be *purely* enantiomeric for the required stereochemistry.
- A pre-biotic environment can't generate homochirality.
- Amino acids don't just have an A and a B prong. Half of the amino acids also have a C prong that winds up getting in the way. They couple in the main chain. Enzymes were needed from the very beginning of the process to make proper folding possible.
- If you had a mixture of amino acids and sugars in the same place and time trying to make sugars, the amino acids have the same alcohol groups that would compete. The amine groups would compete in the same types of reaction and would preclude sugar formation.
- The correct Electron Spin Selectivity (ESS) is needed.
- The folding of primary structures into functional secondary and tertiary structures)along with minimum Gibbs free energy sinks) could not have been anticipated by an inanimate environment.
- Nobody has ever explained higher order structuring (engineering). Neither non-equilibrium thermodynamics nor Gibbs-free energy minimization explains how so many specific functional shapes were prescribed by linear digital Prescriptive Information (PI) [68,69,204]

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- The Levinthal 1.0 paradox asks how nature could have formed the needed sequencing of monomers in a linear chain of nucleosides or amino acids (primary structure) and have it wind up folding into the needed three-dimensional shape (secondary > tertiary structure) to become the required specific enzyme [205,206].
- Foldamers and chaperones are additional enzymes needed to assist the proper folding into the needed three-dimensional shape. But how were *they* produced in a prebiotic environment?
- Translational pausing is critical to protein folding [180,207–210]. Translational pausing is controlled, not constrained, by superimposed, multi-layered coding in the mRNA [180].
- Alignment is not just a covalent bond problem, but a non-covalent spatial interaction problem also. The Levinthal 2.0 paradox addresses astronomical possibilities from which only a very few are usable. In many cases, this is where the Universal Plausibility Metric of life-origin models measures out to less than a ξ of < 1.0. The Universal Plausibility Principle is thus violated [203], requiring peer-review rejection of the model for lack of scientific plausibility. Mere possibility does not make a model scientifically plausible.

4.3. Coded Prescriptive Information Is Not Just Metaphorical

- Nobody has solved the code problem for the sequencing of certain nucleosides, all with the same chemical bonds, into prescribed sequences of polynucleotides.
- No instructions (Prescriptive Information, PI) [68,69,174] exists in an inanimate, prebiotic environment. What was steering and controlling all this chemistry to avoid tar production?
- Nucleic acid prescriptions have to be programmed with arbitrary representational code according to rules. That instructional code then has to be instantiated into a replicable physical matrix in order to generate repeated production in the future. This is especially true for any newly needed enzyme. How did inanimate nature accomplish all this?
- Gene editing (e.g., Crispr) is engineering, not natural science. How were genes edited into useful prescriptions prebiotically?
- Production of the needed fatty acids, glycerol ethanolamine and lipids are all directed and engineered by coded Prescriptive Information [68,69].
- Non-covalent interactions have to all be aligned because Prescriptive Information travels down these channels by electrostatic potentials.

4.4. Membranes

- A huge number of highly specific transmembrane proteins are needed.
- Glycoproteins, transport proteins, cholesterol, glycolipid, peripheral protein, internal protein, filaments of cytoskeleton, integral protein, surface protein, Alpha-helix protein, hydrophobic tails, hydrophilic heads, phospholipids, and highly specific carbohydrates are all needed.
- Lipase and many other enzymes are needed to make a real cell membrane. No enzymes of any kind are present in a micelle or vesicle environment. Not even enough functional peptides are there yet.
- The building blocks of lipids are fatty acids, phosphate, glycerol and ethanolamine. Very few of the incredible number of possible three-dimensional steric lipid formations fit the required bill for any conceivable active transport membrane or form of life to arise. Cell membranes have highly selective pores that allow only certain metabolites in, and preclude others from getting in. Then, there are critical excretory and secretory pumps.
- A bilipid layer micelle is a cartoon of an active transport membrane with highly selective pores. Not just osmotic gradients are required, but an incredible array of essential homeostatic requirements is maintained by cellular membranes in the simplest uni-cellular organisms.
- Outside lipids are different from inside lipids. Very complex layers of lipids exist even in organelles. They are highly organized with undeniably orchestrated functions, not just self-ordered by law or constraint.
- Ionophore pores are highly selective. What exactly does selective mean? The answer to this question is not explainable by any law, constraint or the four known forces of physics. Selection

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has to be active, not passive, for a proto-cell to even faintly resemble life. A cell membrane requires thousands of different lipids and protein-lipid complexes.

- Monoacyl lipids are a catastrophe. Different diacyl lipids are required on the inside from the outside to perform the required proton gradient and pumps.
- Nobody knows how natural law could prebiotically make the outside of the cell membrane different from the inside in a functional sense. An inanimate environment sees no need to arrange the tails and heads so as to achieve function.
- Lynn Margulis' models [211–214] just presupposes organelles rather than explaining their origin. Membranes are critical to organelle function, too.
- How are monoacyl lipids avoided in a prebiotic environment?
- How were all the highly specific protein-lipid complexes made for selective transport.
- How were nutrient ingestion, waste excretion, and secretion channels in the supposed "protocell" developed to make it even resemble a protocell rather than a pathetic vesicle or micelle.
- A proton gradient is needed. How did prebiotic nature achieve that? *Protocells cannot be orchestrated and engineered into existence by mere laws and constraints.*
- Bioengineers have clearly defined the minimum requirements for the simplest protocell to come to life. Of the 15 minimal essential components, absolutely none has been made in a prebiotically relevant environment!
- Chemists haven't even made pure yields of the four basic classes of molecules prebiotically, let alone the compounds of those basic classes.
- The protein-protein interactions alone in a simple yeast cell have 1,079,000,000,000 possibilities. There are only 10^{90} elemental particles in the cosmos!

4.5. The Needed Manufacturing Plants

- Inanimate nature must have had all 20 amino acids (or possibly 22), and only those amino acids, available in the same place at the same time to make most ANY enzyme.
- Even if you have all 20 at the same place and time, how is the cross-linking problem solved caused by half of all amino acids having a C prong? Enzymes are required to keep that from happening. But in order for those enzymes to form, they themselves had the exact same problem.
- 2′5′ dinucleotide contamination prevails. 2′5′ dinucleotides cannot code for protein! 3′5′ dinucleotides are essential for abiogenesis.
- Yet spontaneously formed RNA yields a mixture of 75-85% 2′-5′ dinucleotides. This would have precluded naturalistic abiogenesis, If only 1% were 2′5′, NO functional peptides can be instructed or constructed.
- Each amino acid has to have three nucleotides coding for it. If one out of three has a 2′5′, no amino acid is coded.
- Small interfering RNA (siRNA) is formed from 2′5′ RNA: siRNA stops translation. In RNA, the 2′5′ linkages (30 to 70%) act like siRNA
- Chemists have to store reagents at -112 degrees F (!) to make 3′-5′ dinucleotides
- Nucleobases need protection. The phosphate needs activation.
- To make nucleotides in the lab, glassware must be washed with 3% H_2O_2 . Then, the glasswork requires ten washes with RNAse free water. This could never have happened on early earth.
- Primed RNA has never duplicated more than 10% of itself.
- A hands-off, spontaneous formose reaction is an implausible source of a pure dextro-ribose and RNA. Many of the chemical species generated in controlled laboratory conditions are nothing more than carboxylic acids [215]. To any qualified chemist, a spontaneous formose reaction is not the explanation hoped for.
- You cannot get the moieties needed to do any sort of synthetic chemistry work needed for life to form even when the world's finest synthetic chemists are controlling ~~the~~ all of the many needed processes.
- Dipyranose's interactome has 10^{79} billion potential combinations. There are only 10^{90} elementary particles in the cosmos! Where is this objective reality in the minds of naïve, simplistic thinkers when they argue, "The life-origin problem has largely been solved"?

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- Even if you have all 20 amino acids, they must be separated and isolated.
- The smartest micelle-vesicle researchers cannot design and engineer even an adequate active transport membrane, let alone a real protocell. Any progress in that direction is always proven by Materials and Methods to be teleological (which, of course, we euphemistically try to reduce to "teleonomy.") All of these papers defeat the very purpose for which they were written: to demonstrate the capabilities of naturalistic physicalism. What is demonstrated instead is humanistic creationism. No human agency, . . . no experimental success!

4.6. Heritability

- Inorganic abiogenic Metabolism-First models have no heritability and no way to sustain any accidental "successes," not that a prebiotic environment would have known what a "success" was.
- How would an inorganic or organic composomal reaction sequence have been preferentially preserved, and by what means?
- *Eons of time*
- There's not enough time in 14 billion years, and not enough elementary particles in the cosmos, to overcome relevant probability bounds [216].
- Inanimate nature could not have collected in piecemeal fashion all components through long periods of time. There would be no basis for secondary, passive selection without a superior final product to differentially survive. Organisms first have to be alive to differentially survive best.
- Eons of time is not the savior of abiogenesis theory. Eons of time is its greatest enemy.

4.7. The Contention that "Cells Were Much Simpler Back Then"

How simple were they, asks synthetic chemist Prof Tour [199]?
The simplest holistically "living" cell would have had to manifest right from the beginning:

- DNA replication, repair; restriction, modification
- basic transcription machinery
- Amino-acyl tRNA synthesis:
- t-RNA maturation and modification
- Tremendously conceptually complex Ribosomes
- Ribosomal proteins and their organization and orchestration
- Ribosome function, maturation and modification
- Translation factors
- Controlled RNA degradation
- Protein processing, folding and secretion
- Superimposed, multilayered coding (Superimposed codes of Ontological Prescriptive Information (PIo) [69,204] purposely slows or speeds up the translation-decoding process within the ribosome. Variable translation rates help prescribe functional folding of the nascent protein [180]. Protein folding would have been critical right from the start.)
- Cellular replication is highly prescribed and controlled. It is not just "cell division."
- Intra-cellular molecular transport
- Glycolysis
- Proton motive force generation
- Pentose phosphate pathway
- Lipid metabolism
- Biosynthesis of nucleotides and cofactors
- Minimization of heat release. The need to mitigate chiral-induced spin selectivity to prevent cellular heat stroke.
- Homochirality had to be there from the beginning. Homochirality could not have been developed through time. Any protocell would have burned up without chirality.
- Membrane transport is highly selective and exquisitely tailored to cellular needs.
- Micellar, vesicular and proto-cellular concepts are not immune to such requirements.

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- Excretion of waste, ingestion of nutrients, secretion—are all mediated by a true cell membrane that thoroughly embarrasses any lipid bilayer micelle/vesicle of a supposed protocell.
- No purified reagents, buffers, or catalysts were present in a prebiotic environment. Everything had to be manufactured from the simplest molecules: CH4, NH3, CO2, O2, H2S, sulphate, H2O, formaldehyde, carbonate, formate and cyanide. Many of these needed molecules are lethal to life.
- No source of phospholipids or nucleosides existed in an inanimate environment; no human-designed coupling agents or protecting groups; no H_2O_2 and distilled-water-rinsed and dried flasks; no purified solvents; no vacuum pumps or degassing steps; no ability to arrest or restart reactions when needed; no method of transfer of reagents from one flask to the next for critical sequential steps done in the required order, etc.
- The Materials and Methods in abiogenesis research papers are most often not prebiotically relevant or plausible.

4.8. The Need for Prescriptive Information (PI)

A common contention is that the instructions to organize and orchestrate life came from a template, typically from a ribozyme or other RNA analog (an auto-catalytic RNA-like precursor). The question is, where did the *templated instructions* come from? Mere Clay surface? Since when does mere clay (e.g., montmorillonite) contain formal instructions to do *anything* sophisticated?

A short 200 mer protein has 20^{200} permutations. And that phase space would be racemic. The number of permutations is way larger than 10^{50}. Only a very small percentage of these permutations fold into functional tertiary structures [198]. Thus, most Protein-First models of abiogenesis are statistically prohibitive. But the real questions are, "How did inanimate nature sequence linear digital instructions out of this phase space?" How did prebiotic nature assign formal arbitrary code assignments and meaning to those assignments? What were the scientific mechanisms for achieving transcription and translation? Just two classes of aminoacyl-tRNA synthetases (aaRS's I and II) do not provide adequate answers. These are *not* chemical reaction problems. They are programming delegations. Coding and translation from one language into another are *not* physico-chemical. They are abstract. Biosemiosis can be instantiated into physical symbol vehicles (tokens) within a Material Symbol System [103,171,188,189]. But the coded instructions themselves are abstract, not physical. Prescriptive Information (PI) [68,69,204] cannot be reduced to physicality, non-equilibrium thermodynamic instabilities included.

We have no explanation for the interactome's *conceptual* complexity. To instruct sophisticated function requires abstract concept. Concept is formal, not physical. So is computation. Concept can be instantiated into physicality according to rules and arbitrary code assignments. But concept cannot be mustered by the laws of motion or mere physico-dynamic constraints. Even a protometabolism would have required controls rather than constraints [217–219]. Controls emanate from concept, not fixed redundant law. They are choice contingent. Controls fall into the fundamental category of Choice Causation (CC), not Physicodynamic Causation (PC) [67,220–222]

Materials and Methods invariably prove the opposite of what physicalist abiogenists wanted to prove. Experimental design consistently betrays "investigator involvement." Every reactant is carefully and actively selected at the just the needed stage of reaction sequence. Reactions are steered to desired end-points. While the title of each paper invokes the contention of "natural process," the experimental achievements are all invariably engineered by agent-controlled lab techniques. Exact measurements, deliberate and careful sequencing of reactions and critical removals of reactants at the needed times from the reaction environment are the most common features of agent-controlled experimental design. Neglect of these details, and organic labs become tar factories every time.

5. Conclusions

Much of Tour's emphases precedes this author's main concerns regarding overall prescription and orchestration of protocellular metabolomics [223,224]. Tour deals primarily with very high

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production hurdles of generating even the simplest building block biomolecules, even before peptides and proteins were needed.

The plausibility of all physicalistic life-origin models quickly comes into question, especially when a *concert* of statistically prohibitive spontaneous processes was required.

Non-equilibrium thermodynamic instabilities, Prigogine's dissipative structures, phase transitions, irreversibility, entropy and complexity are all often proclaimed to explain abiogenesis. Support for this perspective usually comes from carefully designed and engineered experiments that produce one, or a few, biomolecules known to be difficult to synthesize. Limited spontaneous inorganic and organic reactions are also cited that *could have occurred* in a prebiotic environment. These terms are appropriate in historical science to describe possibilities that cannot be empirically proven.

Possibility, however, is not Plausibility. Very real probability bounds exist that establish both statistical prohibitiveness [216] and legitimate scientific implausibility [203]. Science has an obligation not to waste grant money, time and resources on implausible models. Hypotheses of mere possibility have to be tempered. Editors and pier reviewers have an obligation to place plausibility constraints on papers arguing for the very *un*scientific, "anything can happen."

Another paper by this same author in this issue emphasizes the need to elucidate how the process of protometabolism was orchestrated in an inanimate environment. This problem becomes especially acute when *active selection* is metaphysically prohibited from all discussion. Some form of active selection would have been required in an inanimate environment to orchestrate and engineer into existence even the simplest protometabolism. Even many biochemical pathways, and their required catalyses, constitute incredibly sophisticated *processes* (e.g., the tricarboxylic acid cycle; or the reverse TCA cycle). We must not only explain biochemical pathway generation, but the integration of all these pathways into holistic protocellular metabolomics. Functional setting of an incredible number of configurable switches was needed. Biochemical circuits had to be orchestrated. Coding schemes, language and messaging are all rule-based, not law-based. Transcription and translation assignments were arbitrary, not militated by law.

It is surprising that something so seemingly subjective as plausibility can actually be measured. The Universal Plausibility Metric [201–203] is extremely valuable in differentiating mere possibility from scientific plausibility. The mere possibility of these models does not equate with their Universal Plausibility Metric. The Universal Plausibility Principle requires peer review rejection of any hypothetical model that measures out with a Universal Plausibility Metric of $\xi < 1.0$.

Physicodynamic Incompleteness [225] and the Formalism > Physicality (F > P) Principle [221,226] both come into play in abiogenesis. The orchestration of life's organization requires crossing the Cybernetic Cut [218,227]. Controls are needed, not just laws and constraints [217]. In the absence of Choice Causation orchestrating all of the conceptually complex and interwoven schemes, the plausibility of a purely physicodynamic abiogenesis translates into religious "blind belief."

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