Origins of Life: Chemistry and Evolution

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Abstract

Progress in understanding the origins of life will be enhanced if models and their predictions are clearly understood and explicitly articulated. Two distinct models can be used to explain the genesis of biopolymers during the origins of life. In one model, which has been pursued for nearly 50 years, RNA is the result of inherent chemical reactivities of prebiotic chemical species. RNA invented evolution. This model enables the prediction that if the conditions of the ancient earth are sufficiently constrained, chemists will discover the direct synthetic pathways that produced RNA. In a fundamentally different model, which is more recent, RNA and other biopolymers are proposed to be the result of prolonged, creative, selection-based changes that occurred during chemical evolution and overlap with early biological evolution. Evolution invented RNA. In this evolutionary model, inherent chemical reactivities are not necessarily relevant to the origins of life and do not predict biosynthesis. These two models are fundamentally different from one another and guide design of very different experimental approaches to test their underlying assumptions. It is currently undetermined which model, or a hybrid of them, is closer to reality.

Introduction

Seeking an account of our origins is a quintessential marker of human curiosity. Today, that quest is focused on the scientific search for the origin of life itself. We now know that the backbones and sidechains of life's essential biopolymers - RNA, DNA, and polypeptide - were fixed in chemical structure at the time of the last universal common ancestor, nearly four billion years ago (1-4), and that they have been held invariant over all biological time and all speciation. Thus, the origins of biopolymers embody the central question about the origin of life. Where did biopolymers and their building blocks come from? What processes converted mixtures of prebiotic small molecules on the Hadean earth (5-7) into sophisticated informational, functional, and structural biopolymers built in living cells by condensing homochiral building blocks into specific sequences with specific linkages?

Two main models have been offered to explain the origins of biopolymers. One model, direct chemical synthesis, was proposed over 70 years ago and is supported by the celebrated Miller-Urey experiment (8). The other model, origins by chemical evolution, is more recent. Here we provide general framework for how chemical evolution can work. We describe and evaluate both models, their assumptions, predictions, strengths, and weaknesses. We compare the models but stress that hybrid models, incorporating aspects of both, are possible. For simplicity, we focus on the origins of RNA, but the discussion relates to other biopolymers as well.

Model 1. Origins by Direct Chemical Synthesis.

A model has been advanced in which RNA first arose on the ancient earth by direct chemical synthesis (9-15). This model envisions *stepwise reactions directed by inherent chemical reactivities.* Direct chemical synthesis starts from small molecule feedstocks that react in serial and parallel synthetic reactions to ultimately produce RNA. In this model, the origins of life is understood through the lens of organic chemistry. Benner and coworkers refer to variations of the direct chemical synthesis model as 'path hypotheses' (12). Within the basic model, there are many variations, with different reactions and ordering of intermediates.

In the direct chemical synthesis model, processes were constrained by specific environmental scenarios of the Hadean Earth (6, 7, 9, 12, 16, 17). Low molecular weight molecules such as water, hydrogen cyanide, cyanamide, formaldehyde, and/or glycoaldehyde spontaneously react to form nucleobases and sugars **(Figure 1)**. Then, intrinsic reactivities of ribose and nucleobases lead to

nucleosides. Intrinsic reactivities of nucleosides and phosphate or phosphite lead to nucleotides. Then, nucleotides combine with each other to form RNA. And finally, RNA, which is capable of catalysis and information storage and transduction, ushers in a distinct and discontinuous phase - Darwinian Evolution (12).

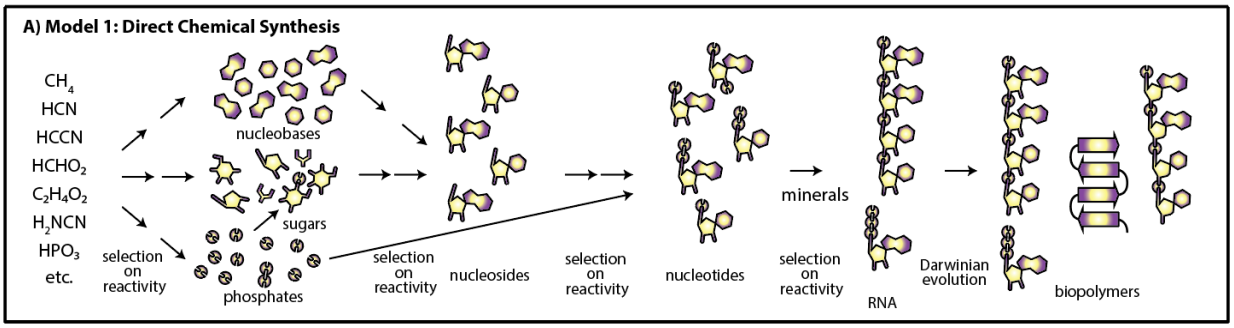

Figure 1. Direct chemical synthesis of RNA, whereby small molecule feedstocks (LH side) enter synthetic pathways that lead to RNA building blocks, then to RNA. This panel was adapted from Benner and coworkers (12). Variations on ordering of the steps and on the specific chemical reactions and intermediates have been proposed.

Some variants of the direct synthesis model assume formation of phosphorylated carbohydrates on the path to nucleosides (18), while others accede the possibility of proto-RNA, a chemical and functional homolog of RNA that was ancestral to RNA. Because the chemical coupling of ribose with cytosine or uracil has proven problematic, some hypotheses link fragments of bases and sugars before the formation of nucleosides (14, 19). Other variations in the specific ordering of steps have been explored. In one hypothesis, precursors of ribonucleotides, amino acids and lipids arose through common chemistry (10). Some variants of the direct synthesis model involve facilitators such as minerals or borate anions (12, 20).

If the direct synthesis model is broadly correct, then important goals of origins of life research are to understand prebiotic conditions and inherent chemical reactivities, and to recapitulate the synthetic pathways that led to biopolymers. If one can know the conditions of the ancient Earth, inherent reactivities, and the probabilities of certain stochastic events, then one can understand and hopefully recapitulate the origins of biopolymers. The RNA branch of this effort was initially led by Orgel and Oro, and has been extended by Benner, Sutherland and others [recently reviewed by Krishnamurthy (21)].

RNA is pre-evolution: The direct synthesis model is discontinuous – an initial era of non-evolutionary synthetic chemistry is distinct from a second era of Darwinian evolution. In this model, RNA origins are pre-evolution (14, 22). Thus, RNA changed the world because it enabled evolution. As stated

by Orgel, “natural selection through replication and mutation was the only mechanism for evolving complex biochemical systems from simpler ones” (13).

Predictions of Origins by Direct Chemical Synthesis.

The direct synthesis model of RNA is inspired by work of Miller (8) demonstrating that organic molecules, including racimates of some biopolymer building blocks, are produced under hypothesized pre-biotic scenarios. Miller demonstrated that simple molecular species such as water, methane, ammonia, and hydrogen combine directly to form biological amino acids such as glycine, alanine, and aspartic acid. The success of this experiment has been interpreted to suggest that most or even all the basic building blocks of life emerged through direct synthesis, and has driven the search for direct synthetic routes to RNA (9-15).

From Miller, we know that inherent chemical reactivities of chemical feedstocks can lead to production of organic molecules that include a racemic subset of biological amino acids on Earth (8). The same applies in outer space; some biological amino acids are found in chondrite meteorites (5, 23). The pathway does not end with monomeric amino acids. Amino acids readily link to form peptide bonds by a variety of mechanisms (24-26). Chemical pathways have been experimentally validated that lead to structures resembling polypeptide. Corroboration of the direct synthesis model would be the discovery of direct synthetic pathways to bases, sugars, nucleosides and nucleotides and polymers. Ribose and nucleobases but not nucleosides or nucleotides are found in meteorites (27, 28).

Weaknesses of Origins by Direct Chemical Synthesis.

Miller-Urey getting blurry. Some weakness of the direct synthesis model have been discussed by Krishnamurthy (21), Shapiro (29) and others. Most fundamentally in our view, is that Miller-Urey results have been misinterpreted. The results of Miller-Urey were interpreted by many to suggest that not only organic molecules (30, 31), but the basic building blocks of life (9-15), can be produced by direct synthesis from small molecule feedstocks. This interpretation has shaped research into the origins of life for seventy years. However, efforts by a broad scientific community have failed to reveal credible direct synthetic routes to common biological species including nucleotides, proteogenic amino acids tyrosine, phenylalanine, lysine, histidine, asparagine, glutamine, cysteine, methionine and arginine (32, 33) or common metabolites such as acetyl coenzyme A, glutathione or phosphorylated sugars.

A nucleotide, which is an oligomer of a nucleobase, a ribose, and a phosphate, is profoundly more complex than any amino acid, with more atoms, more functional groups, more hydrolysis products, more elementary components, more chiral centers, and less stable bonds. The high energy phosphate bond of

a nucleotide has no analog in an amino acid. Proposed direct synthetic pathways for nucleotides are generally different for different types of nucleotides, and are composed of converging branches, each with multiple synthetic steps, in precise order, under different conditions, separated by purifications, and after all that, are not successful. It has been demonstrated that direct synthesis simply does not produce the complex homochiral molecules of biology.

Re-wired. Direct synthesis models require that routes to biopolymers developed during a prebiotic phase and were then re-written by biology. Proposed reaction sequences in synthetic pathways are distinctly different from observed biosynthetic reaction sequences that produce building blocks and biopolymers in cells (21, 34, 35). The orthogonality of direct synthetic pathways and biosynthesis, combined with the absence of direct synthetic pathways for most biochemical species, presents a challenge to the importance of direct synthesis of biological molecules during the origins of life.

Foresight. Foresight is not a property of chemical or biological processes. Direct synthesis models appear to require foresight - gratification is delayed until completion of branching and undulating pathways, with no selection at intermediate stages. Monomers are incapable of maintaining or transmitting polymeric information or performing complex catalysis. The properties of biopolymers are emergent upon chirality and polymerization. Neither fragments of biological nucleosides, nor monomeric nucleosides or nucleotides, assemble as base pairs in aqueous solution.

By contrast, non-canonical nitrogen heterocycles, such as melamine or barbituric acid, form glycosidic linkages with ribose and combine to form linear supramolecular assemblies containing thousands of monomeric paired nucleosides (36). In sum, direct chemical synthesis of RNA requires the establishment of pathways in the absence of a stepwise-driving force. Success occurs only at the conclusion of a long and complex series of disconnected synthetic steps. The ancient earth, unlike modern organic chemists, did not experience an imperative to discover chemical pathways to RNA.

The creation of DNA after RNA, a feature of most direct synthesis models, implies additional foresight. RNA has many useful properties but is chemically labile; the 2’ hydroxyl group of ribose is a nucleophile that catalyzes self-cleavage. Because of its lability, RNA genomes (beyond viruses) are problematic. Nature’s solution is DNA, in which the 2’-hydroxyl of RNA has been replaced by hydrogen atom. DNA is persistent chemically, it has been isolated from mammoths that died over 1 million years ago (37). RNA before DNA implies that Nature, before the invention of genomes, produced a polymer (RNA) with the potential to radically change chemical properties (increased persistence, and decreased structural complexity) via a subtle modification, while maintaining base-pairing and formation of double

helices. Again, gratification (increased persistence of DNA) awaits the conclusion and is not selected for in intermediate stages.

No-go co-evolution. The direct chemical synthesis model is besieged by "chicken and egg" dilemmas; one must place multiple improbable events in chronological order because their simultaneous occurrence appears impossible. What came first, RNA or protein? Information or metabolism? In most [but not all (10)] direct chemical synthesis models, different components and systems have distinct and unrelated origins. For example, phosphorylated sugars can arise by one pathway, nucleobases by another, and amino acids by still another (12). Metabolism is not generally part of these models at all.

In biology, everything is linked to everything, nothing is independent. Biopolymers and metabolism are deeply integrated symbiotic systems that live and die together (38). For example, ATP is required for protein synthesis, which is catalyzed by RNA. ATP is a building block of RNA which is synthesized consumption of amino acids, in reactions catalyzed by proteins. RNA, DNA, protein, and biological metabolism are all impossible without ATP.

Chicken and egg dilemmas vanish in models in which origins of systems and molecules are linked and are integrated from the ground up. If origins are linked, one would expect a building block of RNA, required for protein synthesis, to be the basis of metabolism. Therefore, predictions of direct chemical synthesis models, with distinct origins of various systems, appear to differ from the deep integration and dependencies observed of biological systems.

Continuity. The direct synthesis model assumes a discontinuity between abiotic chemistry and biology. In this model, non-evolutionary direct chemistry produced RNA, which initiated evolution and biology initiated. The chemical and biological eras are distinct and the space between them is discontinuous. The abruptness of the transitions, with essentially no intermediate stages, appears to violate the principle of continuity (39, 40), which stipulates modest, consecutive, contingent, ad hoc, and opportune steps. The continuity principle stipulates many intermediary steps that share characteristics of non-evolutionary chemistry and biological evolution - a continuum between chemistry and biology.

Contingency and Likelihood. It might be that chemists will eventually discover a synthetic pathway to feedstocks, to nucleotides, to RNA. If so, the pathways will be composed of compounded branches, each with multiple synthetic steps under varying conditions, in precise order, interleaved by purifications. In practice, one designs numerous syntheses aimed at achieving the molecular target, and explores different reactants, temperatures, solvents, stoichiometries, and order of addition, and purifies intermediates, then explores again until the target is reached. The phrase 'prebiotically plausible' is retroactively redefined (42, 43) to reagents and conditions that combine to give the target. The model is

constrained by coincidences of multiple events that independently have low probabilities of occurrence such as meteor impacts, volcanic eruptions, large scale movements of materials, and transient interactions of organic compounds with salts and minerals (11, 17, 41). Many of the branches can be characterized as "workarounds" to obtain desired products from available compounds such as adenine and uracil, which are known to be formed from simple feedstocks. The laboratory demonstration of a synthetic pathway must be balanced against contingency and likelihood. We may never know if laboratory synthetic pathways are relevant to the origins of life on the ancient earth.

Model 2. Origins by Chemical Evolution.

If the molecules of life did not emerge from direct chemical synthesis on the prebiotic earth, then where did they come from? We suggest a process of gradual evolution. But what is evolution before biological molecules? In the chemical evolutionary model proposed here, the transformation of chemistry to biology is progressive, incremental, and continuous **(Figure 2)**. Selection was (and is) unremitting and relentless during both chemical, intermediate, and biological phases. Darwinian evolution is a special case of chemical evolution. Complex mixtures of small molecules were sculpted and transformed during continuous chemical selection to yield biopolymers (44-46). In this model the final building blocks of biopolymers are allowed to be fundamentally different from organic molecules accessible by direct synthesis. In this model, the origins of life can be understood by a new synthesis of evolutionary theory and practice with chemical sciences.

The power of evolution to create and sculpt molecules is documented by invention of tyrosine, phenylalanine, lysine, histidine, asparagine, glutamine, cysteine, methionine and arginine (32, 33), and of a myriad chemical variants of adenosine (47). Evolution created 1-, 2-, 6-, 7-, and 8-methyl adenosine, 6-dimethyl adenosine, inosine, 6-isopentenyl adenosine (hydroxylated and unhydroxylated), 2-thiomethylated adenosine variants, 6-glycinylcarbamoyl adenosine, cyclic 6-threonylcarbamoyl adenosine and 2'-O-methyl adenosine, 2'-deoxyadenosine, 2'-O-ribosyladenosine (phosphate), and more.

The evolutionary model assumes the line between chemistry and biology is blurred and indistinct; prebiotic chemistry is continuous with biology. These models map concepts of biological evolution onto chemical processes. For example, in environmental wet-dry cycling: (24, 25, 48-56) (a) a *generation* is a single cycle; (b) *heredity* is information passed from one generation to the next; (c) *information* is associated with chemical composition; (d) *selection* is preferential inheritance of certain molecular compositions; (e) *fitness* is persistence of molecules and specific molecular assemblies; (f) *variation* is spatiotemporal differences in information; (g) an *individual* is a chemically isolated molecular ensemble;

and (h) water is the "*energy currency*" that thermodynamically links and drives reactions. These models integrate evolutionary concepts of continuity (57), lack of foresight (4), exaptation (47, 58), symbiosis and co-evolution (38) into chemistry. Several alternative models of chemical evolution have been proposed (44, 59-62) that have critical features in common.

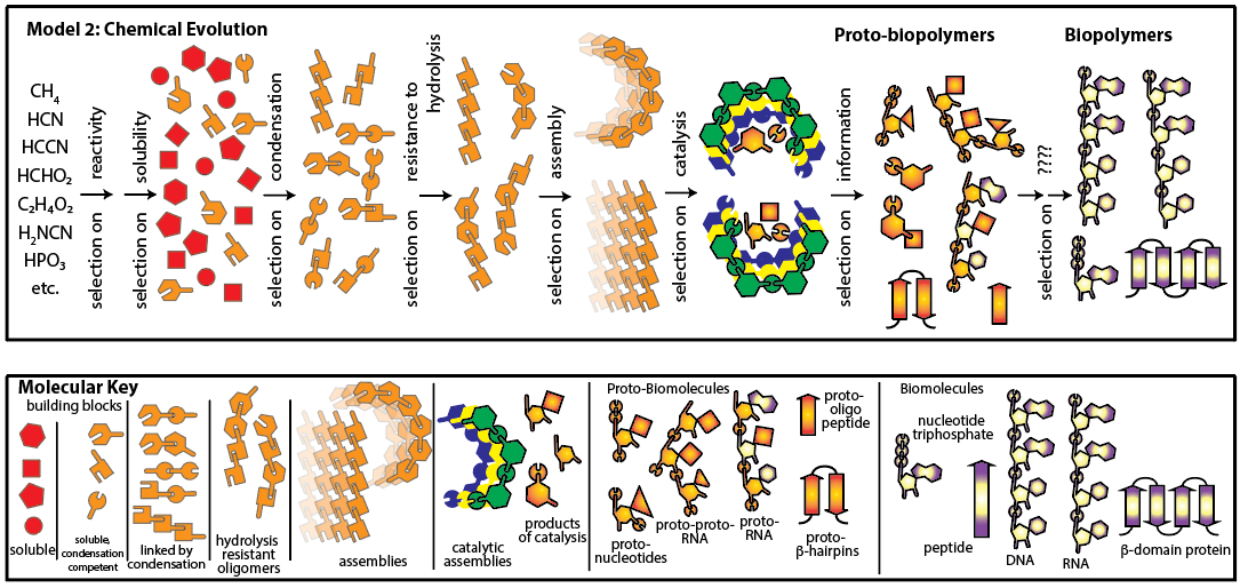

Figure 2. Chemical evolution, in which energy is harvested from environmental cycling and molecules are sculpted by unremitting selection. The basis of selection is fluid, as indicated. This model predicts that biopolymers are composed, at least in part, from building blocks that are not available by direct chemical synthesis. The bottom panel is a key explaining the molecular symbolism. This schematic omits some mechanisms of selection such as compartmentalization. Wet-dry, freeze-thaw or pressure cycling are possible drivers of chemical evolution.

Selection: In the model proposed here, selection is intrinsic to evolution, both chemical and biological. Selection in chemical evolution, like selection in biology, is relentless, yet dynamic and fluid. During chemical evolution, molecules were selected on varying combinations of (i) solubility in water, (ii) ability to link by condensation-dehydration during environmental cycling, (iii) chemical transitions into kinetically trapped (persistent) condensates, such as ester-amide exchange, (iv) resistance to hydrolysis by molecular assembly, and (v) autocatalysis. In this model, intense and mutable selection sparked the genesis of biopolymers.

Creativity: Results of evolutionary creativity (63) are seen in microbial metabolism, tetrapod limbs and primate brains. We suggest that a creative phase of chemical evolution preceded the ongoing creative phase of Darwinian evolution (4). In this model, chemical evolution invented many biological molecules,

which are therefore inaccessible via direct chemical synthesis. Thus, the pathways from chemical feedstocks to biopolymers in models of chemical evolution differ fundamentally from in models of direct synthesis. Molecules created by chemical evolution blur the distinction between pre-biotic and biotic processes.

Chickens and Eggs: Linkage is a norm in evolutionary processes. Advances ripple across and through systems and organisms; linking, for example, the nucleus to the mitochondrion (64), or the wasp to the fig (65). Chemical evolution implies a system in which RNA arose in concert with other biopolymers and with metabolism. Evolutionary models discount proverbial chicken and egg dilemmas because selection is concerted, and changes are coupled across broad fronts. Diverse feedstocks, proto-building blocks, and proto-biopolymers were inter-connected with each other and with primitive metabolism by the chemistry of water and other mechanisms.

Multiple Models: Models of chemical evolution are new and are advancing rapidly (44, 59, 60). Wet-dry, freeze-thaw, and pressure cycling are possible drivers of chemical evolution (66). Hud and coworkers described a model of chemical progression of proto-RNA to RNA, from simple to complex (46). Changes in chemical composition consistent with chemical evolution have been reported during wet-dry cycling (51, 56, 67-70). Unceasing chemical changes and exploration of new chemical spaces has been experimentally authenticated in prolonged wet-dry cycling (71). Baum and coworkers have explored chemical ecosystems (72). Huck has investigated effects of environmental changes on organized reaction systems (73). Mutually catalytic systems (72) and reproducing catalytic micelles (74) have been investigated. The importance of various parameters for chemical evolution (75), including complexity and systems chemistry (76) have been discussed. Oscillatory networks of organic reactions are sustained by compositional heterogeneity, but not by homogeneity (77). Dynamic combinatorial chemistry has been used to discover a variety of functional molecules (78, 79). Auto-catalytic synthesis has been used for selecting functional molecules (80, 81). The majority of work on chemical evolution has taken place over the last 10 years; progress is accelerating.

Predictions of Origins by Chemical Evolution.

Models in which biopolymers are products of co-evolution with each other and with metabolism enable many predictions. The model proposed here predicts that all biopolymers are characterized by: (i) a unified chemistry of polymerization; (ii) thermodynamically unstable, kinetically trapped linkages, (iii) highly sophisticated proficiencies of assembly; (iv) homochirality, a selected by assembly, (v) protection from degradation by assembly; (vi) integration at synthetic, structural, functional and metabolic levels; and (vii) divergence of biosynthetic pathways from inherent chemical reactivities. Chemical evolution

predicts the incredible assemblies and long lifetimes of polysaccharide (cellulose persists for over 5,000 years (82)), of polypeptide (collagen persists for over 40,000 years (83)) and of RNA (with Goldilocks zones of persistence (84)). The unity of biopolymer assembly is illustrated in **Figure 3**.

Chemical evolutionary models envision creativity and innovation before the emergence of biopolymers; in chemical analogy with creativity and innovation in, for example, the biological conversion of fish fins to elephant legs (85) and the conversion of jellyfish nerve nets to human brains (86).

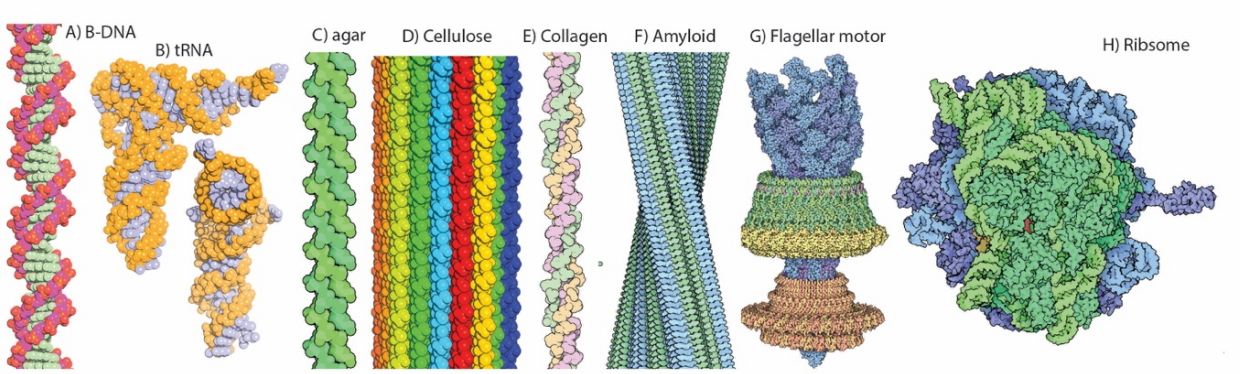

Figure 3. A unity of biopolymer synthesis, folding and protection predicted by chemical evolution. Each biopolymer is synthesized by condensation-dehydration chemistry, has sophisticated proficiency in folding and assembly, and is resistant to hydrolysis when folded and/or assembled. A) DNA, a double-helical polydeoxyribonucleotide. B) tRNA, a complex polyribonucleotide stabilized in part by double helices and in part by more complex interactions. C) Agar, a double-helical polysaccharide. D) Crystalline cellulose, a multistranded polysaccharide assembly. E) Collagen, a triple-helical polypeptide. F) An amyloid fiber composed of a helical assembly of β-sheet polypeptide. G) A flagellar motor, which is a pseudo-symmetric assembly of five distinct polypeptide chains. H) The ribosome, a large non-symmetric co-assembly of around 50 polypeptide chains and over 3,000 deoxyribonucleotides. Some of these images were produced by David S. Goodsell and the RCSB PDB.

The predictions of chemical evolution appear to be consistent with observations of contemporary biology. Among these are the amazing folded structures, assemblies and co-assemblies the characterize biopolymers (polynucleotide, polypeptide, and polysaccharide, **Figure 3**) and the sophisticated assembly by biopolymers of elaborate structures such as DNA, tRNA, cellulose, and collagen. The profound integration of biological subsystems is indicated by the multiple roles for compounds such as ATP, which are central to and inextricable from both biopolymers and metabolic systems (87).Biopolymers are made by unified chemistry (phosphate-mediated condensation-dehydration) and are protected from hydrolysis by folding and assembly (45, 84). Integration is seen in the co-synthesis of biopolymers. RNA makes protein in the ribosome and protein makes RNA in polymerases (87). Integration is also seen in building block biosynthesis - five amino acids are consumed in the biosynthesis of one guanine (21, 87).

Biopolymers and their building blocks are deeply integrated with each other and with metabolic systems, suggesting co-emergence of information and metabolism.

Weaknesses of Origins by Chemical Evolution.

The current model of chemical co-evolution has significant weaknesses. In comparison with direct synthesis models, very little effort has been invested in chemical evolution. Therefore, the mechanisms of chemical evolution are not well constrained. The production of long complex polymers by chemical evolution has not been demonstrated. There are few laboratory examples of evolutionary formation of complex oligomers or polymers from small molecules by chemical evolution. Nor are the molecular mechanisms or duration of chemical evolution fully understood. Moreover, there are no realistic molecular models for the critical phase in which chemical evolution transitions to biological evolution.

Another weakness of this model is that the definition of fitness in chemical evolution models remains partially unresolved and appears more elastic than in Darwinian evolution. Fitness may refer, at some stages of chemical evolution, to the ability of fragile molecular systems/assemblies to persist under hydrolytic stress. At other stages fitness might refer to the ability to tune persistence by folding (84). Although models suggest that the molecular losers of chemical evolution are racemates of esters, thioesters, depsipeptides and thiodepsipeptides (25, 55, 56), other extinct intermediates are not characterized (46).

Summary

We have described two general models for the origins of biopolymers. In one model, biopolymers arose from intrinsic reactivities of prebiotic chemicals on the ancient Earth. This model can lead naturally to an RNA World; chemistry invents RNA then RNA invents evolution. In the second model, prolonged chemical evolution breaks the direct connection of prebiotic chemistry to biology. This model leads to a complex world of many players; evolution invented RNA as one component of an intensely integrated system of symbiotic biopolymers (38). Each of these models has strengths and weaknesses. We do not know which model, or a hybrid of them, is closer to reality. Many studies conducted to understand the origins of life have been guided by the direct synthesis model. Far less has been done to establish experimental methods focused on the evolutionary model. We know one thing for certain - future work directed toward understanding the origin of life will reveal fascinating new chemical phenomena.

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