Biological Polymers:
Origins, Evolution and Significance

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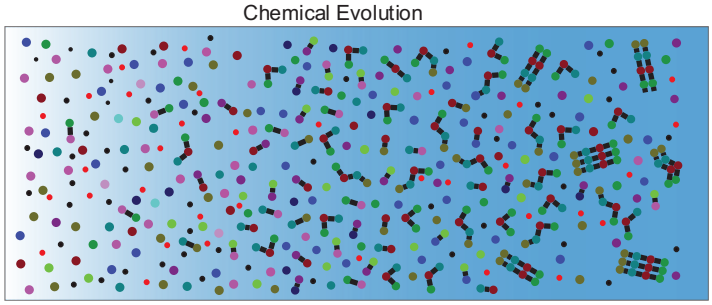
Conspectus (Abstract):



Poorly understood processes on the ancient Earth caused increases in the complexity of organic molecules, creating RNA, DNA, protein, and polysaccharide. In conventional models, extant building blocks, or their close chemical analogs, arose and polymerized via direct synthetic chemistry, to produce RNA. In these models, extant biopolymers retain direct vestiges of prebiotic chemistry and can inform us about the origins of life. In an alternative model, biopolymers might be products of prolonged evolution. In such evolutionary models, chemical species were serially and recursively selected, exapted, reselected, and re-exapted during chemical co-evolution. These later models make the 'gloomy' prediction that biochemistry might have lost many vestiges of prebiotic chemistry. In recent work we and others have begun to determine mechanisms of chemical evolution and have established criteria that allow us to distinguish molecules produced by evolution from those resulting from non-evolutionary physical, chemical, or geological processes. Our approach involves evaluation of shared properties of RNA, DNA, protein, and polysaccharide rather than dissection of any single type of biopolymer. We observe these universal biopolymers to be large, fragile, and elaborate structures with intrinsic abilities to self-protect, and broad arrays of sophisticated functions. All biopolymers are thermodynamically unstable in water. All biopolymers are hyper-functional and undergo switching of function by subtle chemical changes. All biopolymers exhibit homo- and hetero-complementarity that confer ultra-fine control of structure and function. All biopolymers access recalcitrant states, in which assembly confers resistance to hydrolysis and other chemical and enzymatic assaults. Biopolymers engage in mutualism relationships; a cell is an Amazon Jungle of molecules. In sum, biopolymers, in a molecular analogy with the human brain, show hallmarks of evolution; neither chemical nor physical processes, in the absence of evolution, can create the large molecules that dominate biology. The combined data support a model in which chemical species that arose via synthetic

success on the Hadean Earth were subject to a creative chemical co-evolutionary process that ultimately produced biopolymers. We suggest that highly evolved biopolymers, in a smooth and seamless transition, gave rise to Darwinian evolution. The description of biopolymers here will contribute our understanding of the origins of life and for biosignature research.

Key References:

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Introduction

Around four billion years ago, prebiotic chemistry established the molecular keystones of biology, paving a path to life. Chemical and geological processes on the ancient Earth caused increases in the complexity of organic molecules, ultimately creating RNA, DNA, protein, polysaccharides, bilayer-forming amphipaths, and the roots of biology.

The transition of small prebiotic chemical species to complex biological polymers present some of the most fascinating, important, and vexing questions in the fields of chemical and biological sciences. We believe that ultimately humankind will learn to understand, recapitulate and technologically exploit chemical progressions in analogy to those that led to the formation of biopolymers on the ancient Earth. However, we also believe that this understanding will require a challenging integration of chemical sciences and evolutionary theory. In this paper, we seek to explain the utility of this integration, and why is required to fully account for and understand biochemistry.

What are Biopolymers?

Here we consider polypeptide, polynucleotide, and polysaccharide to be three distinct types of biopolymers. Polypeptide is defined here as linear chains of defined-sequence proteinaceous amino acids linked via condensation-dehydration.[1] We follow convention and treat polynucleotide and polysaccharide as separate biopolymer classes.[1] This distinction makes sense functionally, but not chemically, where polynucleotides are seen to be a subset of polysaccharides. Polymers of sugars that form helices[2], or are linked by phosphodiester linkages[3], or are composed of specific sequences,[4] or contain nitrogenous side chains[5,6] are common in biological systems.

Evolution

Over some years we and others[7-9] have worked to understand the possibilities and potential of evolutionary processes in chemical systems. We have proposed that evolution creates molecules with distinctive properties and behaviors; the products of evolution are distinguishable from products of non-evolutionary physical, chemical, or geological processes. Our approach can provide a basis for understanding structures and functions of biopolymers. In addition, we provide tools for evaluating models of origins of biopolymers and can assist with NASA's efforts to infer and observe biosignatures beyond our planet.

The brain is a product of evolution. The brain has function – to integrate and store information and to organize organismal actions and responses through transmission of electrical and chemical signals. The brain is fragile. The structure of the human brain is slowly being unraveled, allowing us to understand its functions.[10] The human brain is composed of nearly 90 billion neurons with precise spatial organization and functions.[11]

The ribosome is a product of evolution. The ribosome has function - to read mRNA and synthesize coded protein. The ribosome is fragile. The structure of the ribosome is directly related to its functions.[12-14] The ribosome is a molecular machine of hundreds of thousands of atoms in precise locations in 3D space,[15] comprising a peptidyl transferase center, a decoding center and a polypeptide exit tunnel.

Evolution leaves footprints. These footprints allow us to know that the human brain and the ribosome are products of evolution. These footprints provide information on evolutionary histories. But what about biopolymers? Do biopolymers display footprints of evolution? Yes. Footprints of biopolymer evolution, defined and explained in detail in the narrative below, include hyper-function, complex structure, relationships between structure and function, homo- and hetero-complementarity, fragility, recalcitrance and Goldilocks recalcitrance, molecular mutualisms, and emergence.

A function is conventionally described as an activity that contributes to organismal fitness. To understand biopolymers, we extend that definition to say that molecular function contributes to molecular fitness, which directly or indirectly enables molecular persistence. Biopolymers are fragile yet have persisted on Earth for around 4 billion years. We say that a molecule is fragile if it is thermodynamically unstable and kinetically trapped. Interstellar polycyclic aromatic hydrocarbons do not have function, are not fragile, and do not demonstrate other footprints of evolution. Chemical and physical and geological processes do not leave evolutionary footprints.

Footprints of Evolution

“Nothing in biology makes sense, except in light of evolution”.[16] We extend Dobzhansky to molecules and argue that nothing in biochemistry makes sense, except in light of chemical evolution (also see[9]). Molecular footprints of evolution help us make sense of biochemistry.

Biopolymers are long, thermodynamically unstable and kinetically trapped (fragile), organic chains synthesized by condensation-dehydration chemistry via phosphorylated intermediates.[17] Evidence for biopolymer evolution is found in collective properties that can be parsed as follows;

(i) Hyper-function and Function Switching
(ii) Complementarity and Self-complementarity
(iii) Recalcitrance: Intrinsic and Intrinsic Control of Chemical Fragility
(iv) Molecular Mutualisms
(v) Emergence

Hyper-function and Function Switching

What is hyper-function? Hyper-function is access to broad landscapes of function. Hyper-function arises from untold iterations of evolutionary selection, exaptation, reselection, and re-exaptation. For example, ancestors of human metacarpus and phalanges (hands) were recursively selected/exapted for a variety of functions before they were selected for propulsion and stability in water (as fish fins), then for terrestrial quadrupedal locomotion, then for climbing, grasping, communication, tactile exploration, etc. This long chain of recursive selection/exaptation provides access to a broad landscape of functions (boxing, writing, driving, swiping left…) that extend beyond those selected during evolution.

Biopolymers are hyper-functional (Figures 1 and 2). Polypeptide (Figure 1) can form α-helical, β-sheet and mixed α/β globular enzymes,[1] and a broad variety of fibers,[18] motors,[19] containers,[20] transporters,[21] sensors,[22] and signals,[23] optical devices,[24,25] adhesives,[26] pores,[27] brushes,[28] and pumps[29]. Globular enzymes have insides and outsides - solvent-accessible surfaces and solvent-shielded interiors. The interiors are ideal for functions such as catalysis of organic reactions. Polynucleotide has an expansive array of functions and is informational,[30] catalytic,[31,32] and structural.[33] Polysaccharide has a broad array of functions and can form single, double, or triple helices,[2,34] worm-like chains,[35] cell walls,[3] insoluble fibers that are chemically robust,[36] and soluble dendrites[37] (Figure 2) that hydrolyze quickly and release chemical energy on demand. Each type of biopolymer is hyper-functional.

A related characteristic of biopolymers is the capacity to remodel structural and functional landscapes via subtle changes in chemical composition. Conversion of polyalanine to polyglycine converts α-helix to intrinsic disorder.[38] Insertion of regularly spaced prolines into a polypeptide abolishes the ability to form α-helices or β-sheets and tips structure toward non-catalytic collagen-type assemblies[39]. Removing one atom of the RNA backbone to form the DNA backbone changes assembly states, helical form, the hydrolytic lifetime, and the catalytic potential[1]. Changing the anomeric linkage of polyglucose from $\beta(1,4)$ to $\alpha(1,4)$ changes the assembly state, hydrolytic lifetimes, and functions. This minor chemical change converts cellulose[36] to amylose.[2] Introducing 10% (1,6) cross-links coverts amylose to glycogen[37].

In sum, biopolymers have hyper-function and remodel their functional landscapes upon subtle chemical change. Narrow attribution of specific functions (e.g., information, catalysis) to a given biopolymer type is unproductive in our view. Chemical species produced by non-evolutionary processes do not have function or hyper-function and do not undergo function-switching. Hyper-functionality and function switching are consistent with origins by evolution.

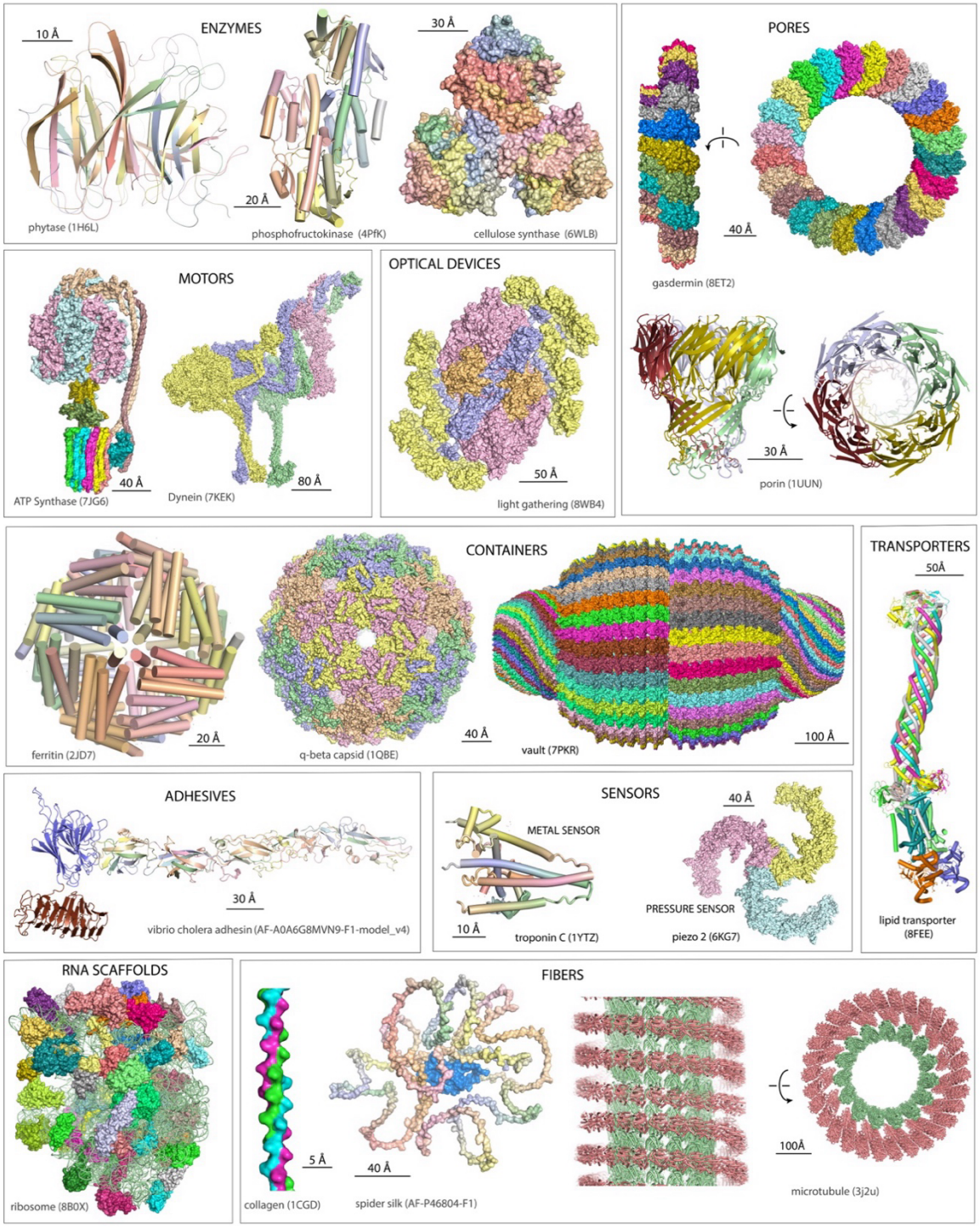

Figure 1. Structures and functions of polypeptide. Polypeptide is hyper-functional and structurally sophisticated. Coordinates were obtained from the PDB or the AlphaFold database and were visualized with PyMol.

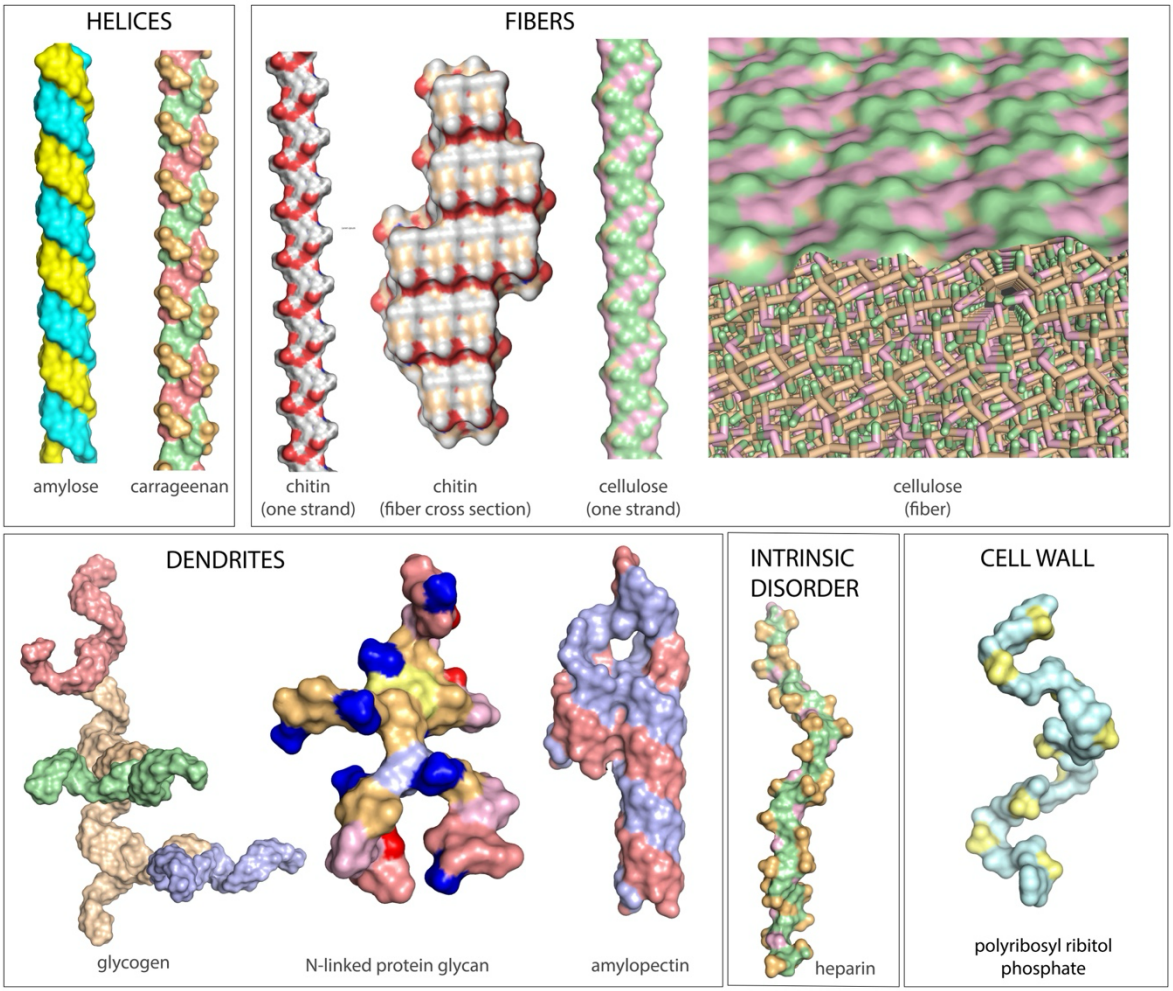

Figure 2. Structures and functions of polysaccharide. Polysaccharide is hyper-functional and structurally sophisticated. Coordinates were obtained from various databases and were visualized with PyMol.

Complementarity and Self-complementarity

Biopolymers exhibit molecular self-complementarity that contributes to fine control of structure and function. The polypeptide backbone is intrinsically self-complementary, as seen in the matched hydrogen bonding donor/acceptor arrays of α-helices or β-sheets.[17] Polyglucose is self-complementary, as seen in assemblies of amylose[2] or cellulose[36] (Figure 2). The sidechains of DNA and RNA are complementary as seen in duplex DNA and structural RNAs.[1]

Biopolymers are hetero-complementary. Proteins can specifically recognize and bind to proteins, DNA or RNA, glycans, and small molecules. An example of complementarity of protein and polysaccharide is seen in Figure 3. The broad competence in self- and hetero-complementarity is consistent with co-evolutionary origins.

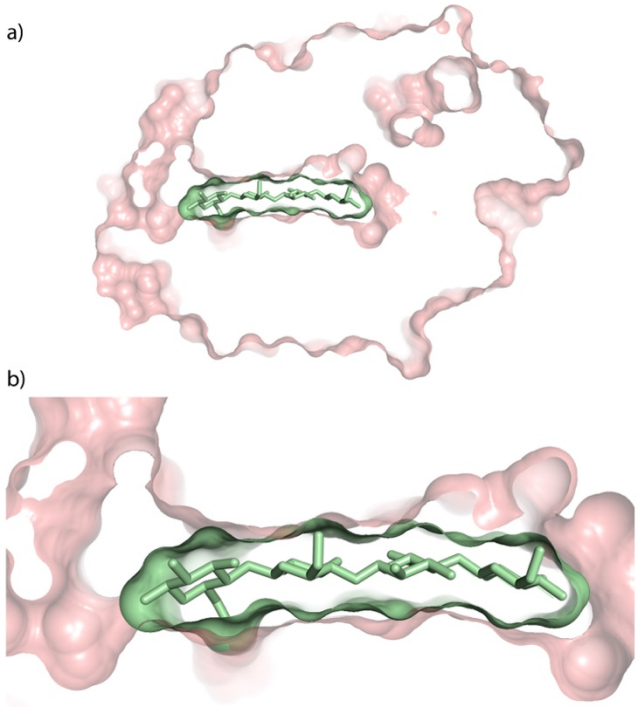

Figure 3. Complementary molecular interactions between the protein cellobiohydrolase I (pink) and the saccharide β(1-4) tetraglucose (green). Van der Waals surfaces are indicated. a) A slice through the entire complex. b) A zoomed view into the complentary protein saccharide interface (PDB entry 5cel).

Recalcitrance: Intrinsic and Extrinsic Control of Chemical Fragility

Evolution has produced thermodynamically unstable polymers that paradoxically dominate much of the chemistry of the Earth. Biopolymers are large, complex, and fragile (thermodynamically unstable and kinetically trapped). Biopolymers degrade spontaneously in aqueous media.[40-46] The negative free energy of hydrolysis (positive free energy for condensation-dehydration, $\Delta G(condense) > 0$) is illustrated in Figure 4. Given sufficient time, DNA, RNA, polypeptide, and polysaccharide degrade in water into small monomeric building blocks. Biopolymers persist in part because they are kinetically trapped. Building blocks are linked by bonds that have high intrinsic activation energies of hydrolysis indicated by $\Delta G^{\ddagger}_{(r)}(int)$ in Figure 4. Kinetically trapped bonds include phosphodiester, peptide, and glycosidic bonds.[43,46,47]

Biopolymers possess mechanisms to modulate kinetic trapping and chemical persistence.[48,49] Biopolymers are proficient at assembly (Figures 1-3), which modulates chemical lifetimes in ways that are not predicted by $\Delta G^{\ddagger}_{(r)}(int)$ (Figure 4).[48,49] To describe this phenomena in general, we appropriated the term recalcitrance and define it as a general tendency of assembly to increase chemical lifetimes of biopolymers.[48,49] The term recalcitrance is taken from carbohydrate chemists[36] who use it to describe the resistance of polyglucose in crystalline cellulose to hydrolysis. Polyglucose in crystalline cellulose is completely unreactive, even to enzymes.[50] The activation energies for essentially any chemical transformation of cellulose include the term $-\Delta G(cryslallize)$ (Figure 4C), meaning that the activation energy for a reaction includes the free energy of decrystallization. Cellulose recalcitrance is a barrier to biofuel production.

Cellulose is not unique. All biopolymers access recalcitrant states. Fibrous proteins and amyloids hydrolyze more slowly and are more persistent than globular domains.[51,52] Disordered linker regions between globular domains hydrolyze more readily than globular domains.[53,54] Assembled collagen has been detected in dinosaur fossils.[55,56] Single-stranded DNA is more vulnerable to chemical and nucleolytic degradation than double-strand DNA.[40,57,58] Folded tRNAs and rRNAs are persistent and robust (Figure 3b) while unfolded mRNAs are labile and fleeting.[49] Polyglucose can persist for hundreds of millions of years[59], or not,[37]

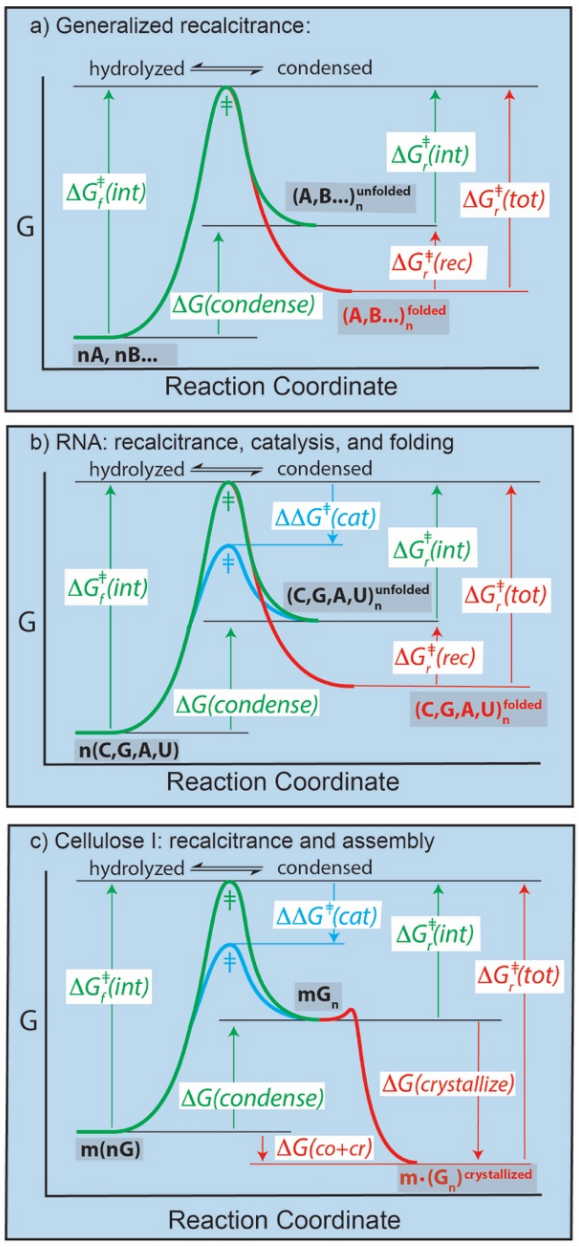

Figure 4. Folding renders biopolymers recalcitrant, with abilities to persist in living organisms and in aqueous environments far longer than predicted by intrinsic chemical lifetimes. a) A generalized schematic of recalcitrance, illustrating the increased activation energy for hydrolysis of folded biopolymers versus unfolded biopolymers. The free energy of condensation of monomer nucleotides (nA, nB…) to form polymers $(A,B…)_n$ in aqueous media is positive [$\Delta G(condense)$>0]. In this scheme hydrolysis occurs in either the folded or unfolded state but at different rates. Intrinsic activation free energies for condensation [green, $\Delta G_{(f)}^{\ddagger}(int)$] and hydrolysis [red, $\Delta G_{(r)}^{\ddagger}(int)$] are indicated. The activation energy for hydrolysis in the folded state $\Delta G_r^{\ddagger}(tot)$ is greater than in the unfolded state by $\Delta G_r^{\ddagger}(rec)$. b) A catalyst or enzyme decreases the activation energies of condensation and hydrolysis by $\Delta\Delta G^{\ddagger}(cat)$. Assembly of RNA causes the activation energy for hydrolysis to increase by $\Delta G_r^{\ddagger}(rec)$. c) Cellulose 1 does not hydrolyze in the assembled state. The activation free energy for hydrolysis includes the free energy of decrystallization.

depending on its assembly state. Biopolymers fall on a continuum; some biopolymers maintain reduced reactivity in assemblies[60-62] while others are essentially unreactive in assemblies.

Goldilocks Recalcitrance. RNA is especially labile,[63] meaning that $\Delta G_{(r)}^{\ddagger}(int)$ (Figure 4) is less for RNA than for other biopolymers. Self-cleavage of RNA involves nucleophilic attack of the 2'-oxygen of the ribose on the adjacent phosphorous atom. The reactivities of 2'-oxygens and the chemical lifetime of RNA are modulated by folding. By simulation and experiment we validated a Goldilocks model of RNA recalcitrance (Figure 5).[49] As experimental models we used yeast-$tRNA^{Phe}$, the *Tetrahymena* ribozyme P4–P6 domain and polyU. For RNAs that fold, local maxima in lifetime are surrounded by conditions of greater lability. For example, RNAs can resist cleavage under conditions where Mg^{2+} folds the RNA. Increasing [Mg^{2+}] beyond the folding threshold or decreasing to less than the folding threshold increases rates of cleavage. Goldilocks regions were observed when RNA was ~95% folded, whereas a control RNA that does not fold, rU20 (polyuridylic acid 20-mer), did not display Goldilocks behavior. We use a Goldilocks model to explain how lifetime landscapes are modulated by specific characteristics of RNAs and by monovalent and divalent cation

concentrations, ligand association, and temperature. RNAs that cannot fold (which are rare) cannot access Goldilocks self-protection. Self-cleaving ribozymes are exempt from Goldilocks behavior because their folding increases rates of cleavage. We propose that Goldilocks recalcitrance was a selectable trait of biopolymers in an early Earth environment, where assembly and reactivity was modulated by factors such as metals, small molecules and water activity.

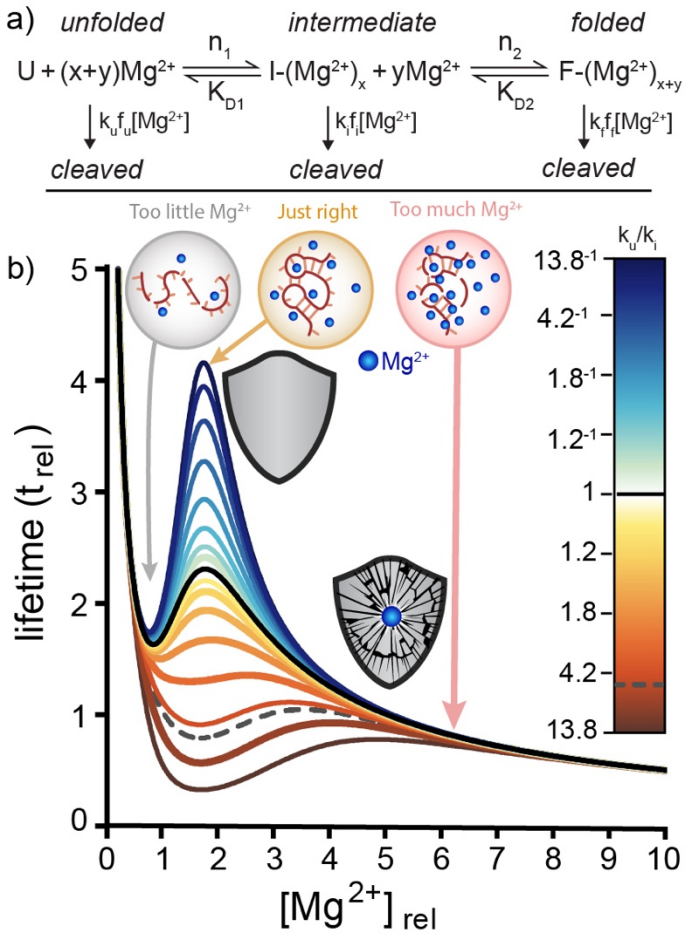

Figure 5. RNA recalcitrance shows Goldilocks peaks of protection. a) In a three-state mechanism, unfolded RNA converts by one transition to an intermediate and by a second transition to fully folded. RNA converts from unfolded to intermediate to folded with increasing [Mg^{2+}]. Unfolded RNA is cleaved with a rate constant k_u, the intermediate is cleaved with a rate constant k_i, and fully folded state is cleaved with a rate constant of k_f. b) In this simulation, k_i/k_u was varied while other parameters were fixed. The black line represents lifetimes when $k_i = k_f$. The dashed line represents the lifetimes when $k_i = k_u$. The most pronounced Goldilocks peak is observed when $k_i < k_f$. The color bar on the RH side indicates k_i/k_u. (adapted from Guth-Metzler, Nucleic Acid Res, 51, 3529 2023).

Hetero-Recalcitrance. One biopolymer can confer recalcitrance on another. Nucleic acids are recalcitrant when bound by proteins. Mutual recalcitrance is the basis of enzymatic and chemical footprinting of DNA-protein or RNA-protein complexes.[64-66] Reactive chemical probes are used to map interactions between a broad variety of nucleic acids and proteins. Mutual recalcitrance explains the differential reactivity of bound and free biopolymers in the presence of nucleases and a broad variety of chemically reactive species including hydroxyl radical, dimethyl sulfate, and lead acetate. Regions of nucleic acids that interact with protein are more recalcitrant than unbound regions. We believe that hetero-recalcitrance was an important mechanism of co-evolution of biopolymers in the evolutionary lead-up to Darwinian processes.

Catalysis, Templating, and Recalcitrance. Biological systems have evolved incredible control of chemical reactivities and can manipulate both the activation energies and net free energies of any given reaction, in isolation of all other reactions. Enzymes stabilize transition states and decrease activation energies by $\Delta\Delta G^{\ddagger}_{(f)}(enz)$. In contrast to enzymes, recalcitrance can decrease a reaction rate in one direction without affecting the rate in the reverse direction. The reaction coordinates in Figure 4 do fully reflect all mechanisms of control. Allosterism,

templating, and other mechanisms modulate enzymatic activity. By contrast, recalcitrance increases the thermodynamic stability of specific species and modulates reactivity in one direction only. An extreme example of recalcitrance involves cellulose. For this reaction the assembled state is completely unreactive; $\Delta\Delta G(rec)$ is equivalent to the free energy of assembly biopolymer function. In our model of the origins of life, fine control of rates of chemical reactivity contributed to survival of the 'fittest' polymers during chemical evolution.

Molecular Mutualisms

We argue that evolutionary concepts can help us explain and understand biochemistry. Mutualisms are an example of that explanatory power. Formalisms developed by biologists for describing mutualisms on levels of cells, organisms, and ecosystems apply to biopolymers and other biological molecules and can help biochemists understand their structure, function, and origins.[67]

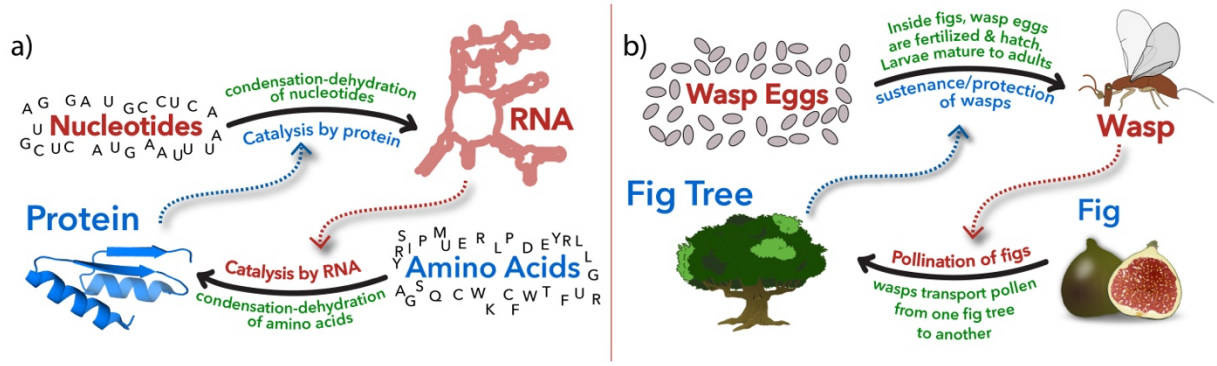

Figure 6. Mutualisms benefit partnering species. a) Molecular mutualism. Proteins make RNA and RNA makes protein. b) The fig-wasp mutualism. The fig depends on the wasps to pollinate fig flowers and initiate seed production. The wasp depends on the fig for nourishment and production of offspring (adapted from Lanier, J Mol Evol, 85, 8 2017).

A mutualism (Figure 6) is a persistent and intimate interaction that benefits partnering species.[68,69] A mutualism is reciprocal exchange; a species proficient in obtaining certain benefits confers those on a second species, which reciprocates by conferring different benefits on the first species.[70] Mutualisms are everywhere in the biosphere and are fundamentally important in ecology.[71] All species on Earth participate in mutualisms. Mutualisms can increase productivity, abundance, and temporal stability of both mutualists and non-mutualists in food webs.[72] Mutualisms (i) sponsor co-evolution, (ii) foster innovation, (iii) increase fitness, (iv) inspire robustness, (iv) are resilient and resistant to change, and (v) involve partners that are distantly related with contrasting yet complementary proficiencies.

Mutualisms were previously understood to operate on levels of cells, organisms, ecosystems and even societies and economies. The eukaryotic cell is a culmination of mutualism between simpler prokaryotic cells.[73-75] The majority of land plant families are mycorrhizal. This plant-fungi mutualism is traceable to the origins of land plants.[76] Flowering plants such as the fig (Ficus spp., Moraceae) and insects such as the fig wasp (Agaonidae, Chalcidoidea) form obligate mutual relationships (Figure 6B).[77] The wasp depends on the fig for food and the fig depends on the wasp for pollination. Pollen-bearing female wasps initiate seed

production in the fig by delivering pollen. The fig provides each wasp larva with a fig seed, which is consumed by the wasp.

We established a model in which formalisms describing mutualisms on levels of cells, organisms, and ecosystems apply to molecules.[67] We call these relationships molecular mutualisms. For example, biopolymers are synthetically interdependent. RNA synthesizes protein in the ribosome and protein synthesizes RNA in polymerases. Mutualisms are seen in hetero-recalcitrance. Biopolymers protect each other from chemical assault. Mutualisms are seen in function and assembly. Proteins and peptides promote folding and functions of RNA[78-83] and vice versa.[84,85] Without protein-based pores and pumps, bilayer compartments are physically and biologically untenable.[86] A cell can be understood as a consortia of molecules in mutualism relationships; an Amazon Jungle of molecules (described by the interactome). Mutualisms drive co-evolution, thereby resolving 'chicken and egg dilemmas'[87] in the chronology of RNA and protein origins.

Molecular mutualisms can also be manifested as covalent linkages between different classes of biopolymers. For example, glycans covalently linked to proteins comprise 50% or more of the total molecular weight of a glycoprotein. Protein glycosylation, which is a result of co-translational or posttranslational modification, affects protein solubility, folding, and aggregation. Lipidation of peptides and proteins with long-chain lipids, which is a common endogenous post-translational modification in today's biology, has been shown to induce membrane association. Lipidation can modify the biophysical properties of the covalently-linked peptides, including their water solubility, self-aggregation propensity, and thermal stability.

Molecular Mutualisms before Biopolymers. In evolutionary models of proposed here, molecular mutualisms predate biopolymers. In these models, mutualisms were important among molecular ancestors of DNA, RNA, protein and polysaccharides, providing mechanisms of biopolymer co-evolution. Mutualisms between molecules in a prebiotic environment would have expanded the chemical landscape and the space for chemical selection. We hypothesize that ancestral mutualisms involved hetero-recalcitrance, chaperoning of folding or solubility, catalysis and auto-catalytic cycles.

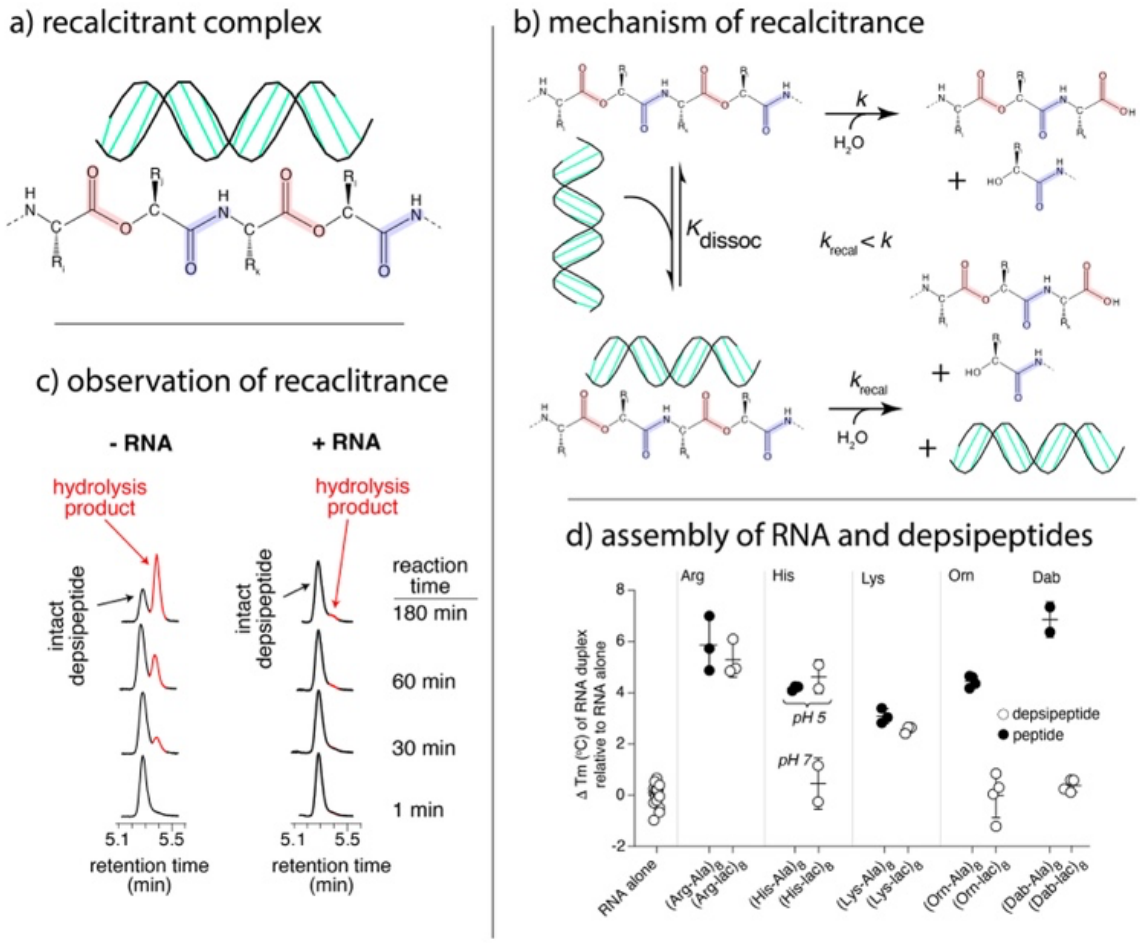

Figure 7. Hetero-Recalcitrance and Molecular Mutualism in a model prebiotic system. a) A schematic diagram of a complex of a cationic depsipeptide and an RNA duplex. b) A kinetic model of hetero-recalcitrance in which the rate of hydrolysis of a depsipeptide is reduced by association with RNA. c) An experimental demonstration of hetero-recalcitrance showing that the rate of hydrolysis of a depsipeptide is reduced by association with an RNA duplex. This image shows HPLC traces (270 nm) of intact and cleaved depsipeptides at various time points in the presence or absence of the RNA duplex at 37C. d) Association with cationic depsipeptides increases the stability of the RNA duplex to thermal melding. The RNA duplex is (5'-rCrGrCrUrArArArUrCrG-3' and 5'-rCrGrArUrUrUrArGrCrG-3', 2.5 uM strand). The depsipeptides (100 uM) are in buffered solution (10 mM phosphate, 100 mM NaCl, pH 7.0 or 10 mM acetate). Ac acetyl, Aba acetamidobenzoic acid was appended to the N-termini to increase UV absorbance. (adapted from Frenkel-Pinter, Nature Commun, 11, 3137, 2020).

We have experimentally confirmed mutualisms between RNA and proto-peptides (polyesters and depsipeptides), which form easily in dry-down reactions. Depsipeptides contain backbone ester linkages in place of some amide bonds, and are thought to be the ancestors of peptides.[88,89] Depsipeptides form readily under mild dry-down of mixtures of hydroxy acids and amino acids.[88,90-94] Ester linkages enable the formation of amide bonds through a process of ester-amide exchange.[88,91,92] α-Hydroxy acids can be incorporated ribosomally during translation to generate depsipeptide and polyester, supporting the notion that depsipeptide and polyester could have been primordial versions of today's proteins.[95,96] Hydroxy acids are produced together with amino acids in model prebiotic reactions,[97] are found together in some

meteorites,[97,98] and can combine to form oligomers >20 residues in length in mild dry-down reaction conditions.[88,90-94]

Our mutualism experiments show that cationic depsipeptide interact with RNA duplexes and stabilize them[99] (Figure 7). Various cationic depsipeptides increase the Tm of RNA duplex melting. Depsipeptides containing positively-charged proteinaceous amino acids (Lys, Arg, or His) promote RNA duplex stability to a greater extent than depsipeptides containing non-proteinaceous prebiotic building blocks (ornithine, 2,4-diaminobutyric acid, or 2,3-diaminopropionic acid). The ineffectiveness of depsipeptides containing ornithine and 2,4-diaminobutyric acid in increasing RNA thermal stability is attributed to more facile intramolecular O,N-acyl transfer reactions in these structures compared to the positively-charged proteinaceous amino acids (Arg, Lys, or His), leading to the degradation of ornithine- and 2,4-diaminobutyric acid-containing sequences during thermal melting. RNA in-turn can stabilize and extend the chemical lifetimes of cationic depsipeptides. Specifically, association with an RNA duplex increased the observed lifetime of a depsipeptide by up to ~30-fold. A single strand of RNA increased the depsipeptide lifetime, but to a lesser extent (about five-fold). These results, combined, are a demonstration of the possibility of primitive mutualism interactions between proto-biopolymers, where both gain fitness by association.

Creativity and Emergence

Evolution is a creative force.[100] To paraphrase Dobzbansky:[101] *Evolution is a creative adventure. It is creative in the sense that an artist is creative. It brings about absolute novelties, constellations of genes which did not exist anywhere before. Evolutionary creativity, as artistic creativity, involves a risk of failure, miscreation, which in the biological world means death, extinction*. Evolutionary creativity has followed a chronology.[102] As noted by Jacob, “the really creative part in biochemistry must have occurred very early.”[103] Creativity in multicellularity was relatively late. Creativity in neurology is ongoing.

Evolutionary creativity is related to emergence. Emergent phenomena cannot be predicted. A multi-component complex system can exhibit emergent properties that cannot be deduced or anticipated from properties of isolated system components.[104] The ribosome, the spliceosome, and the mitochondrion are emergent inventions that demonstrate evolutionary creativity.

Biopolymers are emergent. Their structures and functions cannot be anticipated from the behaviors of non-polymerized building blocks. Structures and functions of proteins (Figure 1) cannot be recapitulated with monomeric amino acids or predicted from properties of amino acids. Monomeric amino acids can act as hydrotropes,[105] but cannot assemble into elaborate structures. The structures and functions of polysaccharides (Figure 2) cannot be predicted from the properties of monomeric sugars. Monomeric glucose does not form fibers or dendrites. The structures and functions of RNAs cannot be predicted from the properties of nucleotides. Monomeric nucleotides in aqueous solution do not form base pairs.[106] The emergent properties manifest in biopolymers are consistent with predictions of origins via evolution. Only evolution could have created emergent biopolymers.

It has been said that evolution can give *the appearance* of design.[107] Evolution creates complexity, functionality and emergent phenomena that naively seem to have been designed for a purpose.[108] Such appearance does not mean that evolution acts with intentionality or foresight; it does not. Evolution has no more consciousness or intelligence or foresight than do gravity or electromagnetism.[107]

Biopolymers are stamped by the footprints of evolution (Figures 1 and 2). These extraordinary molecules share many attributes. Biopolymers are fragile but are protected by recalcitrance and are wildly abundant over the Earth. They engage in intense mutualisms. Their functions are transformed by subtle chemical changes. Yet each type of biopolymer is structurally and functionally distinct from the others. The totality of biopolymer proficiencies is greater than the sum of the parts. Structures and functions of biopolymers in combination are emergent and cannot be recapitulated with isolated biopolymer types. Replication requires both a protein polymerase and nucleic acid template. A simple model to account for the emergent properties of biopolymers is their creation via co-evolution in a common milieu in which control via hetero-recalcitrance over hydrolytic degradation and other chemical assaults was a unifying early selective principle.[48] Non-biological species such as polypropylene and quartz are technologically useful but do not exhibit emergence, are not created by evolution, and therefore are readily distinguishable from biopolymers, ribosomes, and brains.

Darwinian and Non-Darwinian

Where did biopolymers come from? Darwinian evolution, as generally understood, requires biopolymers and so could not have originated them. Genes are made of biopolymers and encode biopolymers. Genes are the units of biological heredity. Template-directed replication of genes with variable sequences and selection of the traits conferred by those genes are the basis of Darwinian evolution.

The dependence of Darwinian evolution on biopolymers appears to be irreconcilable with origins of biopolymers via evolution. Darwinian evolution requires sophisticated polymerases and so could not have originated polymerases. However, the evolutionary origins of biopolymers, as described in the preceding narrative, appears undeniable. Is there a defensible model that can explain and predict a creative progression from simple molecules of prebiotic chemistry to complex biopolymers? Can we envision a manner of evolution that could produce biopolymers? We believe the answer is yes.

We have described an experimental system and theoretical model in which biopolymers arose by non-Darwinian evolutionary processes.[109,110] This model integrates chemical sciences and evolutionary theory; chemical evolution transitions seamlessly into Darwinian evolution, as a creation of chemical evolution. Our working definition of chemical evolution is continuous chemical change with exploration of new chemical spaces and avoidance of equilibrium.[109] We propose that large and diverse populations of small molecules, proto-oligomers and proto-biopolymers were iteratively and recursively selected and sculpted and exapted to produce the building blocks and biopolymers that enabled Darwinian evolution, and survive in extant biology.

To follow evolution in complex mixtures during wet-dry cycling, we investigated changes over wet-dry cycles of a mixture containing 9 components. Analysis of reaction products was monitored by HPLC, NMR, and LC-MS.[109] The rate of chemical change was greatest in early cycles, then declined, and stabilized at a non-zero value for the duration of the cycling. The data are consistent with a model in which the system continuously evolved and did not converge, or reach a steady state, throughout the course of the experiment.

In sum, we present a model, and certain data to support it, in which life on Earth was preceded by, and sponsored by, sustained chemical evolution. It seems likely that the chemical evolutionary process that led to biology is a special case of a general phenomenon. Chemical evolution, once understood, might have the potential to transform chemical sciences in general. This model opens the exciting possibility of applications of directed chemical evolution to a broad range of applications ranging from pharmaceuticals to material sciences. If an evolutionary process produced incredible molecules such as RNA and protein, then humankind can gain advantage by understanding and redirecting that process.

Our evolutionary model maps elements of biological evolution onto chemical processes. We say that during environmental wet-dry cycling: (a) a generation is a single cycle; (b) heredity is information passed from one generation to the next; (c) information is associated with non-random 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 molecules to each other and to the environment. During the origins of life, a ‘system’ harvested energy from the ‘surroundings’ and invested it in creating biopolymers. In this model biological molecules are products of evolution and are not necessarily represented in abiotic inventories on the ancient earth. Chemical evolution does not require biological molecules or template-directed replication.

Models and Data

Models of direct chemical synthesis of biopolymers have dominated origins of life research over the last half century. In these non-evolutionary models, extant building blocks, or their close chemical analogs, arose[111] and polymerized via direct synthetic chemistry on the abiotic Hadean Earth.[87,112-114] These non-evolutionary models assume that combinations of fortuitous geologic, organic and inorganic processes produced biopolymers, which have remained fixed over all of evolution.

The essence of these models was expressed in a recent review,[115] which states, “…the core structure of nucleic acids appears to be a natural outcome of non-biological chemical processes…approximately 4.36 ± 0.05 billion years ago.” In these direct synthesis models, biology incorporated and has maintained prebiotic building blocks and polymers; extant biopolymers provide information on prebiotic chemistry. As noted in a second review,[114] “…extant life, despite billions of years of evolution, has retained some direct vestiges of its prebiotic chemistry.”

These models generally assume that all evolution is Darwinian. The assumption of a single kind of evolution is the basis of RNA World models. "…Darwinian evolution is the only mechanism by which matter can organize itself to give properties that we value in life."[115]

By contrast, in evolutionary models, chemical species that arose via synthetic success on the Hadean Earth were sculpted, elected, exapted, resculpted, reselected, and re-exapted during creative chemical co-evolutionary processes. In this process, biopolymers, were selected not for a specific function, but for hyper-functionality (Figures 1 and 2). In this model, hyper-functional biopolymers eventually gave rise, in a smooth transition, to Darwinian evolution. The evidence that biopolymers are products of chemical evolution eclipses our lack of complete understanding of mechanisms of chemical evolution. The evidence for biopolymer evolution cannot be discounted because we do not fully understand mechanisms of that evolution. Historically, the importance of the distinction between data and models is illustrated by the rejection of strong evidence of plate tectonics by many geologists in the early and mid 20th century in part because they could not imagine a model for movement of continents.[116] The evidence for biopolymer evolution is sufficiently strong that Darwinian evolution should be discounted as the sole mechanism by which matter can organize itself and evolve.

Evolutionary models of biopolymer origins are departures from previous models of direct chemical synthesis. Evolutionary models make Orgel's 'gloomy' prediction[117] that biochemistry might have lost vestiges of prebiotic chemistry. Chemical evolution may have substantially erased and re-written prior prebiotic chemistry. If so, how do we confront the origins of life? What experiments should we do? In fact, evolutionary models of biopolymer origins are experimentally accessible, for example by wet-dry or freeze-thaw cycling. There is much to be learned about effects of duration, feeding, seeding, library composition, cycling temperature and frequency, low frequency perturbations (seasons), etc. A lack of direct connection biochemistry to prebiotic chemistry should not deter us from constructing and experimentally testing evolutionary models. Currently we do not know if it is possible to recapitulate specific steps in chemical evolution as it occurred on the early Earth. Human labor probably cannot do what evolution can do. We can hope to someday understand what evolution has done and influence what evolution will do. We believe that new models integrating evolutionary theory into chemical sciences will lead to advances in prebiotic chemistry and in chemical sciences in general. A change of paradigm seems positive and exciting.

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