ACCOUNTS
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A Modular Hierarchy-Based Theory of the Chemical Origins of Life Based on Molecular Complementarity

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CONSPECTUS

Albert Szent-Gyorgyi once defined discovery as seeing what everyone else sees and thinking what no one else thinks. I often find that phenomena that are obvious to other people are not obvious to me. Molecular complementarity is one of these phenomena: while rare among any random set of compounds, it is ubiquitous in living systems. Because every molecule in a living system binds more or less specifically to several others, we now speak of "interactomes". What explains the ubiquity of molecular complementarity in living systems? What might such an explanation reveal about the chemical origins of life and the principles that have governed its evolution? Beyond this, what might complementarity tell us about the optimization of integrated systems in general?

My research combines theoretical and experimental approaches to molecular complementarity relating to evolution from prebiotic chemical systems to superorganismal interactions. Experimentally, I have characterized complementarity involving specific binding between small molecules and explored how these small-molecule modules have been incorporated into macromolecular systems such as receptors and transporters. Several general principles have emerged from this research. Molecules that bind to each other almost always alter each other's physiological effects; and conversely, molecules that have antagonistic or synergistic physiological effects almost always bind to each other. This principle suggests a chemical link between biological structure and function. Secondly, modern biological systems contain an embedded molecular paleontology based on complementarity that can reveal their chemical origins. This molecular paleontology is often manifested through modules involving small, molecularly complementary subunits that are built into modern macromolecular structures such as receptors and transporters. A third principle is that complementary modules are conserved and repurposed at every stage of evolution.

Molecular complementarity plays critical roles in the evolution of chemical systems and resolves a significant number of outstanding problems in the emergence of complex systems. All physical and mathematical models of organization within complex systems rely upon nonrandom linkage between components. Molecular complementarity provides a naturally occurring nonrandom linker. More importantly, the formation of hierarchically organized stable modules vastly improves the probability of achieving self-organization, and molecular complementarity provides a mechanism by which hierarchically organized stable modules can form. Finally, modularity based on molecular complementarity produces a means for storing and replicating information. Linear replicating molecules such as DNA or RNA are not required to transmit information from one generation of compounds to the next: compositional replication is as ubiquitous in living systems as genetic replication and is equally important to its functions. Chemical systems composed of complementary modules mediate this compositional replication and gave rise to linear replication schemes.

In sum, I propose that molecular complementarity is ubiquitous in living systems because it provides the physicochemical basis for modular, hierarchical ordering and replication necessary for the evolution of the chemical systems upon which life is based. I conjecture that complementarity more generally is an essential agent that mediates evolution at every level of organization.

What is an adaptive chemical system and how could one arise? Solving this problem will illuminate not only prebiotic evolution but the nature of living systems in general. I argue that the answer is to be found in ecological systems of compounds that form hierarchically organized modules based on molecular complementarity and that employ compositional replication. These properties are just as typical of all biotic chemical systems as are linear replication

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molecules and catalytic networks but far less studied. Understanding the principles of evolution by modular complementarity suggests mechanisms by which natural selection prunes away huge numbers of possibilities to direct evolution toward the increasingly integrated systems. This pruning process transforms evolution from a probabilistic near-impossibility into an almost certain consequence of our Earthly chemistry. Complementarity principles may be "scale-free", applicable to every level of organization from molecular to societal.

Oddly, my route to these conclusions is firmly based in the philosophy of science, which has defined my approaches to information flow as embodied in the so-called "Central Dogma of Molecular Biology" and the relative merits of mechanistic reductionism versus systemic holism. I was serious enough about history and philosophy of science to obtain my Ph.D. in the subject, with Thomas Kuhn, the promulgator of the theory that science progresses by revolutionary paradigm shifts that punctuate periods of "normal" problem-solving science.[1] Kuhn taught me that progress in science is facilitated by skeptically challenging assumptions and focusing on anomalies dismissed by others. I vowed to apply these lessons to my own scientific research and was fortunate enough to obtain a postdoctoral position with Jonas Salk that permitted me to do so.[2] The position with Salk had an unexpected bonus: I worked next door to Leslie Orgel and Art Weber for three years, often borrowing their equipment and learning first-hand about their breakthroughs in the prebiotic origins of nucleotides and sugars.

But I was already engaged in origins of life research by the time I began my postdoctoral work with Salk. My interest in the subject began with a question about information flow in biological systems. A student in a lab for which I was a teaching assistant wanted to know how I reconciled the Central Dogma with the immunological differentiation between "self" and "nonself". The Central Dogma is usually stated as genetic information flows from DNA to RNA to protein, but Francis Crick, the Dogma's inventor, actually stated it in the opposite way: once information gets into proteins, it cannot get out.[3,4] I objected to this formulation because it is nontestable.[2,5] Moreover, the immune system "reads" proteins to determine whether they are antigenic, which means that the information in these proteins is somehow compared with the genetic information determining "self". How is this possible? Mathematically modeling the problem with a new formalism called "Petri nets" specifically invented to model information flows in computer systems suggested that the simplest solution to the self–nonself problem was to abandon the Central Dogma in favor of a

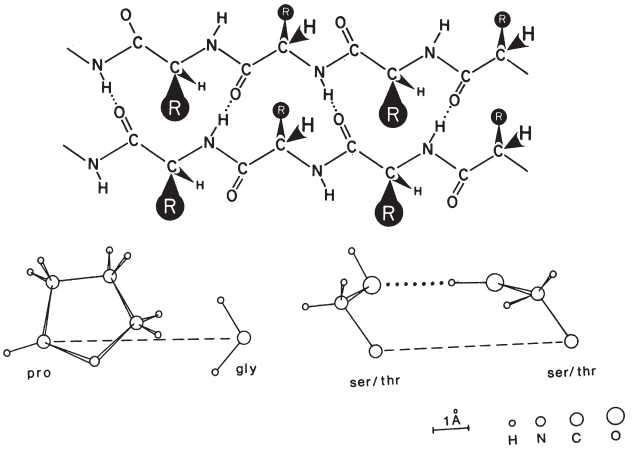

FIGURE 1. (top) Parallel β ribbon showing amino acid side chain (R group) alignment. (bottom) Amino acid side chain interaction across a β ribbon illustrated by proline–glycine and threonine–serine pairs. Specificity of side chain interactions results in genetically encoded "antisense peptides".[5–11]

concept in which proteins should be able to encode complementarity proteins (e.g., antigen-encoding antibody) through a process that I called (after DNA base pairing) "amino acid pairing".[5] Structural modeling revealed that the amino acid side chains on β ribbons align (Figure 1). Side chain alignments limit the possible pairings to a small subset of all the permutations so that any given amino acid has only one or two others with which it can interact. To make a very long story short, the paper that I wrote on this topic was savaged repeatedly by reviewers, took four years to get into print, and eventually became one of the foundational papers in a field now known as antisense peptides developed by myself, Meckler, Biro, and Blalock.[6–11] Strands of sense and antisense RNA or DNA do encode complementary peptide sequences that specifically bind to each other just as complementary polynucleotides do. Evidence also hints that protein-to-protein and possibly protein-to-RNA "back-translations" are carried out in the immune system by so-called "transfer factors".[12]

Because amino acid pairs are genetically encoded,[13] amino acid pairing naturally led me to ponder the origins of the genetic code itself, and before I knew it, I was engaged in origins of life research. Most importantly, I had entered origins of life research through the doorway of molecular complementarity (base and amino acid pairings), and I have viewed the field through the lenses of complementarity ever since. This perspective has opened new doors on small molecule complementarity.

What is molecular complementarity? Molecular complementarity is the stereospecific, reversible binding of two or

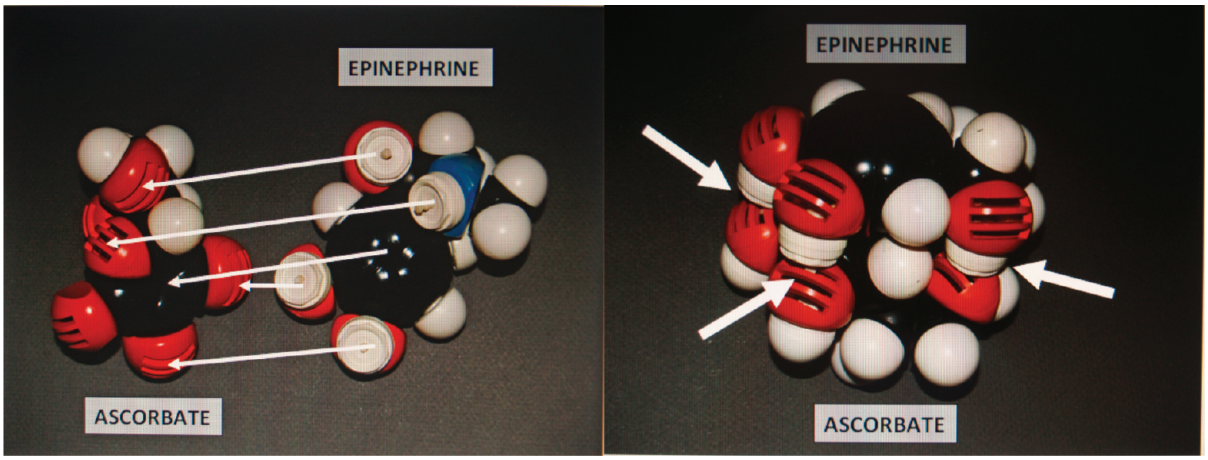

FIGURE 2. (left) Epinephrine and vitamin C (ascorbic acid). Arrows show four hydrogen bonds and $\pi-\pi$ overlap bonds. (right) Resulting structure showing three of the hydrogen bonds.[20,22,44]

more molecules achieved by a combination of hydrogen, ionic, and $\pi-\pi$ overlap bonds augmented by van der Waals attraction and solvent exclusion.[14] Molecular complementarity is therefore dependent on the fitness of the solvent to support reversible interactions (on Earth, water), as well as on solubility, concentration, and temperature.

Why study molecular complementarity? Mainly because I'm a pattern seeker. I noticed as an undergraduate that molecular complementarity was ubiquitous among macromolecules associated with life. My textbooks described base pairing in polynucleotides, enzyme–substrate and receptor–ligand specificity, antigen–antibody complementarity, and the self-assembly of viruses and ribosomes. More recent molecular biological research has revealed the astounding fact that every molecule in a cell interacts specifically with others, creating networks now called "interactomes". Every aspect of cell function turns out to be structurally organized through molecular complementarity. And all of this was (and still is) described as if it is obvious that nature should behave this way.

It was not obvious to me. One does not observe such ubiquitous molecular complementarity in random mixtures of compounds, so why in mixtures of cellular compounds? How did life evolve to take advantage of molecular complementarity so that the two are virtually synonymous?[15]

I worked backward. Standard evolutionary reasoning assumes that each species is a modification of previous ones so that we can trace their lineages through their morphological paleontology. Geneticists have adapted the paleontological idea to tracing genetic lineages. Might there be, I wondered, yet another level of paleontology embedded in the molecules of life that reflects selection criteria at work from the earliest prebiotic chemistry?

I started my investigations of this conjecture on two parallel fronts. One was to explore whether the origin of the genetic code could be explained by coselection among amino acids (or peptides) and codons (or anticodons). While the idea that amino acids might bind directly to codons or anticodons was not new, the idea that amino acid pairing might act as a second selective pressure in the formation of the code was, and I and others have provided evidence for both kinds of selection.[6–9] I have also proposed, and provided some evidence for, the idea that homochirality in amino acids and sugars arose as a result of the origin of the code itself, rather than earlier in the origins of life as most people have assumed.[16,17]

The second front I explored was small molecule complementarity, which had almost completely been ignored at the time. I assumed that the textbook cases of complementarity that we all studied (DNA, antigen–antibody, enzyme–substrate, receptor–ligand) had to have evolved by selection for smaller molecule complementarity. If so, then the molecular paleontology of such small molecular selection should be just as much a part of modern systems as were the amino acid pairs hidden in the genetic code. In collaboration with Fred Westall, I focused on the then "hot" topic of cotransmission. Cotransmission was a phenomenon observed by neurobiochemists during the 1970s in which pairs of neuroransmitters or hormones (or both) were found to be co-stored and co-released by neurons and other organ systems, For example, norepinephrine was often found with ascorbic acid (vitamin C) (Figure 2) or opioid peptides such as the enkephalins (Figure 4, below) both in neurons and in the adrenals, a phenomenon I will discuss in more detail below.

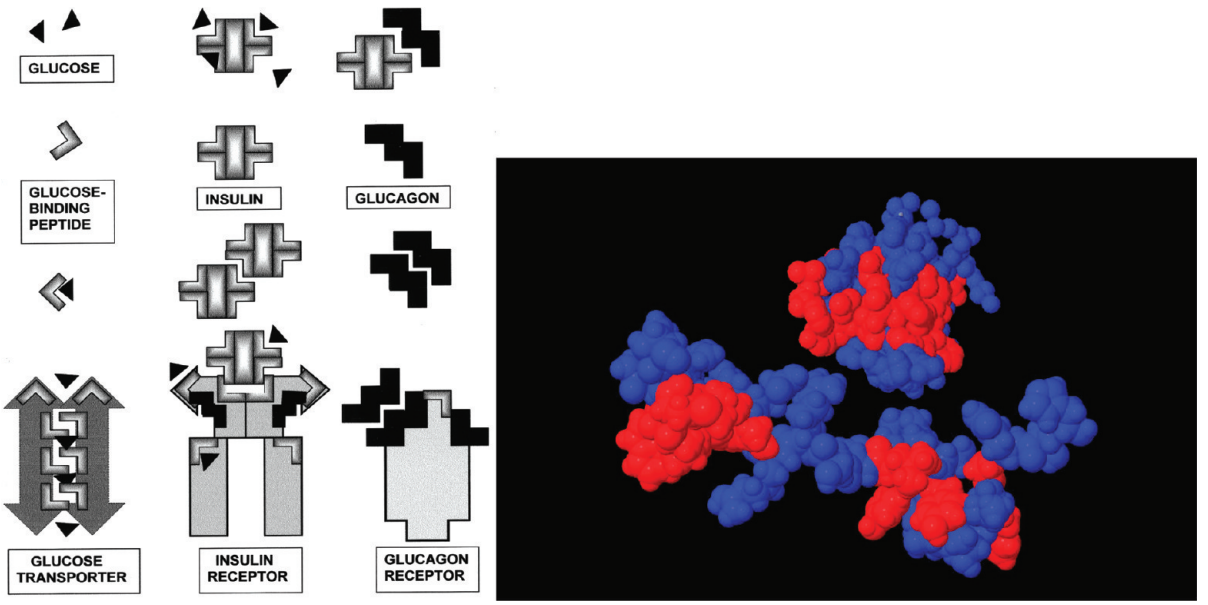

FIGURE 3. (left) Glucose metabolism molecules evolved from a network of highly conserved, molecularly complementary modules. Insulin has six glucose-binding modules homologous to ones found in the insulin receptor and glucose transporters. Insulin self-aggregates, and insulin-like regions are found in the insulin receptor at insulin-binding sites,[29,30] which contain glucose binding modules.[33,34] Similarly, glucagon self-aggregates and glucagon-like regions exist at the glucagon binding sites of the glucagon receptor. Also, insulin and glucagon are complementary,[28] but their cocrystallized structure has never been determined. (right) NMR solution structures of insulin (top) and glucagon (bottom) displaying hydrophilic (blue) and hydrophobic (red) residues. Glucagon's hydrophobic regions may form a pocket for those of insulin.

Sometimes cotransmitters behaved as antagonists, sometimes synergistically. Evolutionarily, pairs of cotransmitted compounds were often functionally and structurally associated even in bacteria and yeast. Again to make a long story short, we found that small molecules that functionally alter each other's activity in biological systems almost always bind to each other and, conversely, small molecules that bind to each other will usually modify each other's biological functions,[18−22] an observation that may have profound physiological and pharmacological implications.[23]

Moreover, evidence suggests that such small molecule complementarity has provided the basis upon which evolution has built more complex physiological systems. Russell Doolittle, who I met while at Salk, proposed during the late 1970s that proteins evolve by modular accretion.[24] Functional peptide regions that are genetically encoded become duplicated and linked to give rise to a small protein, which in turn may swap modules with other proteins. That model for bootstrapping complexity in biological systems stuck with me. I added considerations of molecular complementarity into the mix. For example, I asked myself how I would evolve a regulatory system for glucose metabolism. In modern cells, the peptide insulin acting on the insulin receptor permits glucose to enter cells through glucose transporters where it can be utilized for energy or stored as glycogen; another peptide, glucagon, working through the glucagon receptor, permits glycogen to be broken back down into glucose. How could such a complex system evolve under prebiotic conditions, stepwise, from simple precursors? The simplest solution was to have an insulin-like molecule regulating glucose's concentration by directly binding it up, creating a buffered system of storage and release. Because insulin is hydrophobic, it could ferry glucose through lipid membranes. I began experiments but quickly found that several investigators had already proven that insulin has multiple glucose binding sites.[25−27] Moreover, glucagon, which antagonizes insulin, binds directly to insulin (they even cocrystallize!),[28] and we have preliminary evidence that glucagon binds specifically to glycogen, possibly acting as a weak enzyme. Structure and function seemed to be related through the molecular complementarity of the components at every level of the system.

Donard Dwyer stimulated my next forays into molecular paleontology. Dwyer proposed that self-complementary peptides might give rise to their own receptors.[29,30] Insulin self-aggregates, so following Dwyer, I hypothesized that the insulin receptor should contain insulin-like regions at its binding sites. Homology searching verified this hypothesis,[31,32] and experiments proved that insulin binds to these insulin-like receptor domains.[33,34] Using our paleontological

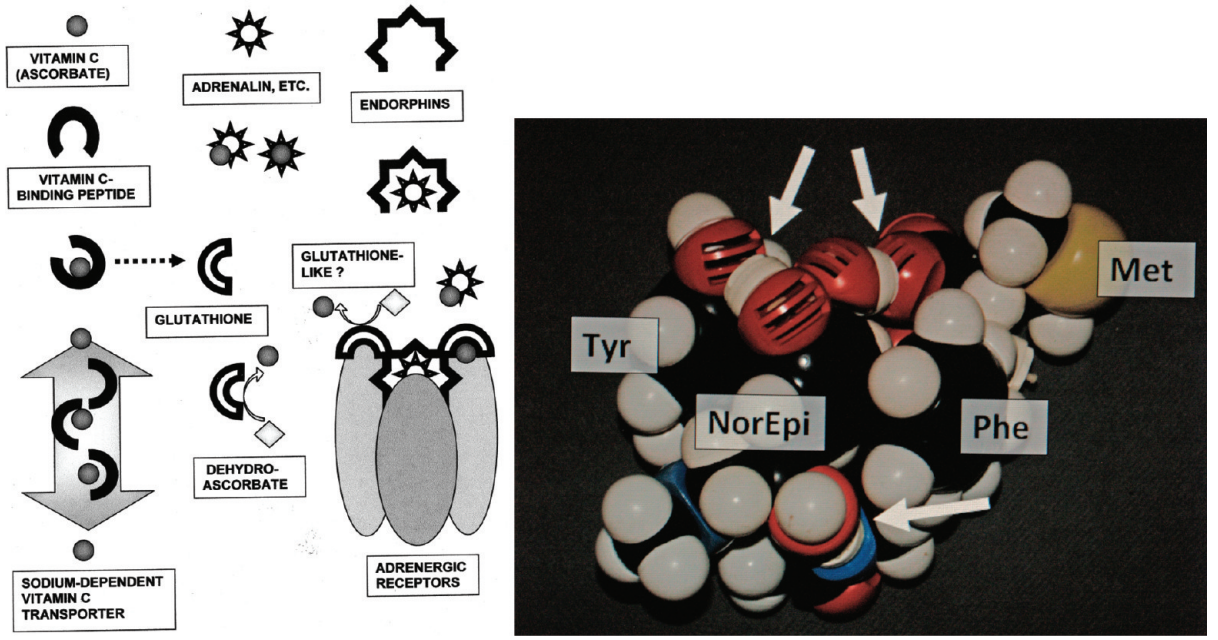

FIGURE 4. (left) Adrenergic compounds bind to ascorbate (vitamin C) and opioids (e.g., enkephalins and endorphins),[20] creating a network of molecularly complementary modules. Oxidized ascorbate (dehydroascorbate) is recycled into ascorbate by the peptide glutathione. Glutathione-like ascorbate-binding peptides are found in sodium-dependent vitamin C transporters and adrenergic receptors and function like glutathione to recycle ascorbate.[38,39] Adjacent to this glutathione-like region is an endorphin-like region that binds adrenalin. (right) Model of norepinephrine (NorEpi) binding to met-enkephalin (Tyr-Gly-Gly-Phe-Met) based on NMR study.[20] Five hydrogen bonds (three shown by arrows) are supplemented by $\pi-\pi$ overlap bonding between the NorEpi, Phe, and Tyr rings.

reasoning, we also conjectured that these insulin-like regions of the insulin receptor might act as glucose binding sites. They do.[33,34] Our model provides a novel mechanism for tuning insulin activity to glucose concentrations. Beyond that, I have proposed that transporters may have evolved in an identical way. The family of glucose transporters is characterized by having a series of insulin-like glucose-binding modules within their transport cores.[35] So understanding the principles of modular complementary can yield valuable insights of physiological and pharmacological relevance (Figure 3).

I have proposed a similar story for the evolution of catecholamine receptors such as those for epinephrine (fight or flight responses) and norepinephrine (neurotransmitter). As noted above (see Figure 1), we had shown that ascorbic acid, the main cellular antioxidant, binds to catecholamines.[20,22] In the absence of this binding to ascorbic acid, catecholamines will oxidize within minutes, but when bound, the complex is stable for days. Since human blood serum has enough ascorbate to bind 80–90% of circulating catecholamines, muscle physiologist Patrick F. Dillon and I wondered what the physiological effects might be. We unexpectedly discovered that while ascorbate has no measurable effect on smooth muscle contractions, when added to any submaximal dose of catecholamines, it increases the effectiveness of the dose and its duration of activity up to 10-fold![36–39] The mechanism appears to result from the adaptation of small molecule complementary modules to building up complex macromolecules. Catecholamine receptors have ascorbate binding sites.[38,39] Binding of ascorbate to catecholamine receptors causes allosteric changes in receptor structure enhancing catecholamine activity. Thus, the receptors have evolved to make use of the ascorbate–catecholamine complex. Moreover, these ascorbate binding sites are homologous to glutathione, an intracellular peptide that recycles oxidized ascorbate, and also to conserved regions of the ascorbate transporter,[35–39] again suggesting that evolution has reused a common peptide module for diverse purposes in different proteins. Finally, the adrenergic receptor appears to include complementary modules selected from opioid peptides, which also act as catecholamine enhancers.[20] Thus, catecholamine receptor function is integrated into structure by several types of complementarity (Figure 4).

A molecular paleontology of small molecule complementarity adds significantly to our understanding of the evolution of chemical systems by providing several key components: a means of naturally selecting among molecules

during evolution; a mechanism for stabilizing and buffering the resulting systems; ways of generating increased functional diversity; and perhaps most importantly, the ability to organize and replicate such systems.

One of the most exciting theoretical developments that occurred while I was toiling away at my chemical investigations of molecular complementarity was Stuart Kauffmann's digital and mathematical explorations of how complex systems could self-organize.[40] The problem with Kauffmann's models of emergent systems, from my perspective, was that chemical systems do not behave like his do. A paper by the economist and psychologist Herbert Simon[41] provided clues as to how to address chemical self-organization.

In order to explain his theory in a nontechnical way, Simon compared two watch makers who adopt very different strategies in assembling their watches. Both make watches composed of a thousand parts. Assume it takes one minute, on average, to add each part. The first watchmaker tries to assemble the entire watch in one continuous effort, which will take him seventeen hours if he does not stop at any time. If he stops, the pieces come apart and he loses all of his work. Since he must stop to help customers, eat, relieve himself, and sleep, this first watch maker completes very few watches during his lifetime. The second watch maker uses a different strategy. She assembles her watches into stable subunits of ten parts that take only ten minutes to complete. Interruptions result in an average loss of five minutes worth of work. She assembles her subunits into larger stable units comprised of ten subunits each, which are also stable unless she is interrupted. Finally, this watch maker assembles her units of 100 parts into the final watch. She assembles a completed watch every few days. As Simon wrote, "The lesson for biological evolution is quite clear and direct. The time required for the evolution of a complex form from simple elements depends critically on the number and distribution of potential intermediate stable forms. In particular, if there exists a hierarchy of potentially stable `subassemblies', with about the same span, *s*, [i.e., the number of parts required to form each stable subunit] at each level of the hierarchy, then the time required for a subassembly can be expected to be about the same at each level – that is, proportional to $1/(1-p)\exp s$", where p is the probability of interruption during assembly.[41−43] The implication of Simon's model is that we should therefore expect evolution to be characterized by the selection of semistable modules arranged in a hierarchical fashion.

Simon's analogy has limitations. Simon, like Kauffmann, did not address what natural evolutionary processes produced his semistable modules. This is where I realized that molecular complementarity comes into play.[44,45] Molecular complementarity is a naturally occurring process for selecting stable modules from which complex systems can be organized. Not all molecules available in the environment play a role in living systems. Before one can "build a watch", one needs first of all to select the components that will participate in building the "watch". Then one needs a means to concentrate and self-organize these components.[44−47] These are the key roles that molecular complementarity plays in evolution.

Oddly, Simon overlooked a major benefit of stable subsystems, which is to increase the probability of evolution by ruthlessly pruning nonstable aggregates.[48] Simon assumed that both watchmakers knew how to make a watch: they were omniscient creators. But science cannot accept such intelligent creation. Nature, left unconstrained, presumably explores all permutations of molecular elements available. In order to make Simon's analogy more "natural", we must substitute for his omniscient watchmakers two of Richard Dawkins's "blind watchmakers" who are also clueless about how to make a watch.

To simplify this part of our analysis and for the sake of dealing with numbers that we can comprehend, let us assume a watch has only 25 parts. Our clueless, blind watchmaker faces a problem much more complex than the one Simon set his original watchmakers. This new watchmaker cannot simply assembly 25 parts in one sitting to make a watch because he does not know their order of assembly or how they fit. Rather he must explore every possible permutation of the 25 parts, which is to say 25!, or about 1.55×10^{25} possibilities! Moreover, because he's a random assembler who cannot learn from experience, he has to explore all these permutations every time! Clearly, such a process would never succeed.[48]

But an equally blind, random watchmaker who uses complementary modules would succeed astoundingly quickly! Assume our clueless, blind *modular* watchmaker makes her watches in modules of five parts that are stable at the end of each building session. Assume also that all other permutations of the five parts are unstable. Stable five-element modules could be built by exploring only 5! possibilities, or just 120 permutations in just two hours, or all five sets of elements in 10 h. Then she would then need to explore the 5! possible permutations of these five modules (120 possibilities) over another 2 h, exploring in all only 720 permutations. The difference between 720 and 1.55×10^{25} permutations is so huge that it makes the impossible highly

likely![47] (Obviously, the savings are not as great as I have just stated for a real molecularly complementary system because the specificity of module building is not going to be perfect and some nonfunctional modules will also be stable, confusing final assembly. Still, savings should be extremely substantial and would explain how life could emerge probabilistically and robustly here on Earth and elsewhere in the universe.)

Complementary module building within complex systems, in sum, can prune out huge numbers of possibilities at each step of hierarchical assembly. In general, the greater the number of pieces and the more modular steps involved in the process, the more efficient the process becomes. Analyzing naturally occurring modular hierarchies for rules of optimization might therefore have vast implications not only for understanding the evolution of life but also for the most efficient design of chemical, technological, and even human systems.

Complementary modularity can increase the rate of evolution compared with purely random processes in two additional ways as well. First, complementarity stabilizes the components against hydrolysis, oxidation, photolysis, and other destructive effects, thereby acting as a form of chemical natural selection.[44−48] This protective effect has been demonstrated for combinations of ascorbate−catecholamine,[36−39] glycylglycine−glutamate,[48] insulin−glucagon,[49] and maleic acid−urea.[50] Mutually aggregating molecules are more likely than others to participate in evolutionary "watch building" processes. Second, complementarity-based modules can self-assemble and duplicate themselves so that our module-building, blind, clueless watchmaker need not randomly explore all of the possibilities every time she wants to make a new watch. Thus, both stabilization and replication lower the local entropy of the system. Entropy is related to information. And so we find ourselves back at the problem that got me involved in origins of life research in the first place, which is information theory. Is all information stored in DNA and RNA, as Crick's Central Dogma implies (and modern RNA-world scenarios state explicitly), or can substantial information be encoded in other molecules associated with chemical and living systems?

Modern information theory is predicated on probabilities such as those that have just concerned us. Schroedinger, Brillouin, Mahulikar, and Herwig and others have variously identified the storage of information with negative entropy, also known as negentropy[51] or syntropy.[52] The basic concept stems from an inversion of Claude Shannon's classic work on information theory in which he identified the amount of information needed to specify a system as a function of the entropy of that system. As a system becomes more ordered, the amount of information required to specify it decreases because the system itself is storing information through its organization. The local decrease in entropy, or negative entropy, can be considered a direct measure of stored information. This information may, alternatively, be described as the decrease in Gibbs free enthalpy of the system or as the amount of order introduced into a probabilistic system (syntropy). By fostering the formation of hierarchies of stable modules within chemical systems, molecular complementarity acts to decrease the local entropy resulting in the storage of information as order or organization. Or, as Paul Weiss wrote, what distinguishes any system from a probabilistic ensemble of components is that the system is *less than the possible sum of its parts* − quite the opposite of the way we usually phrase the emergence of new properties in complex systems.[53] The calculations made above with regard to our dumb, blind watchmakers suggest that huge amounts of information can be stored through natural selection for hierarchically organized networks of complementary modules.[54]

The concept that networks of molecular interactions can store information of biological relevance is critical to my work. Doron Lancet is among the first scientists to appreciate that such interactions can also transmit information, even in the absence linear polymers such as RNA or DNA. Lancet's basic idea is that compositionally diverse aggregates of molecules, or "composomes", could have formed by molecular affinity and replicated by simple fission, just as do lipid vesicles.[55] One can appreciate the basic concept by dividing the contents of a bowl of multicolored M&Ms. For any reasonable number of M&Ms, the distribution will be quite similar to the original. Variations in components may be replicable and have evolutionary advantages.[45,47] Thus, if we replace Simon's watch model, in which each component of the watch is a unique part, with a model of prebiotic systems that is much more chemically accurate in having multiple copies of every compound, then we can imagine such systems as being bowls of mutually attracting modules (M&Ms!) that can replicate by fission (Figure 5). Such compositionally based replicators have recently been characterized (see Mann's essay on "Systems of Creation" in this volume).

In 2005, Lancet, Victor Norris, I, and several other origins-of-life explorers recognized the similarities in our approaches and produced an integrated theory[45,47,56] We realized that compositional replication is ubiquitous in modern living systems. Ribosomes are randomly assorted during

FIGURE 5. "Self Assembly 3" by Robert Root-Bernstein 2005. An illustration of how molecular complementarity produces compositional aggregates that can grow and replicate.

replication. Modular assemblies that are present in small copy numbers, such as, Golgi apparatus, vacuoles, and actin filaments, and enzyme assemblies[57] are broken down into their modular components just prior to cell division, the components randomly distributed to the daughter cells, and the organelles reassembled within the daughter cells. *De novo* synthesis of the components during cell growth provides additional components for the modules for all of these assemblages, which are incorporated by means of their chemical complementarity. Thus, molecular complementarity mediates compositional replication as well as genetic replication within biotic systems.[45,47,56–59]

Our integrated theory also emphasized that the emergent properties could only arise within a complex geochemical enviroment or ecology. While molecular complementarity prunes the possibilities that result in information-containing modular organization, the possibilities must all be present before they can be pruned. Thus, we have summarized our novel integrative theory in two statements: (1) everything that could happen (in terms of chemical reactions) during the origins of life did happen; (2) life is adapted to its environment because it evolved in tandem with its chemical ecology.[45,47,58,59]

So to sum up, I return to my perhaps naïve undergraduate question as to why molecular complementarity appears to be ubiquitous in living systems but nowhere else in nature. The answer seems to be that molecular complementarity provides a natural means of selecting, concentrating, stabilizing, and producing buffered, homeostatic modules from which hierarchically organized systems can emerge in the most efficient conceivable manner, capturing a maximum of entropy as information and providing mechanisms (compositional and linear replication) for chemical "inheritance".

Where to from here? My research has convinced me that the reductionist philosophy of science and hypothesis-driven experimentation have severe limitations. The synergistic effects of glucose–insulin, norepinephrine–enkaphalin, and ascorbate–epinephrine could not be predicted from their *individual* properties. Since emergent properties cannot be predicted, combinations and mixtures must be explored simply to see what happens. Permutations to be explored may be limited by using the concept of molecular paleontology to identify chemical combinations that living systems seem to have selected for functional adaptations. We must, in short, "complexify" our experiments, not simplify them, if we wish to understand living systems.

Beyond the origins of life, I am interested applying the concept of complementarity to other levels of organization. For example, if living systems are completely integrated molecularly complementary systems, then might the immune system itself have evolved to protect the integrity of that integrated system from noncomplementary components that threaten it? Might this complementarist perspective reveal novel aspects of pathogenicity and immune function?[60,61] Indeed, might the concept of complementarity be expanded in a scale-free manner beyond molecules to the evolution of multicellularity, symbiosis, animal communication, animal and human cultures, indeed, to ecological, social, political, economic, and other forms of interaction mediated by nonrandom interactions between agents? This possibility excites me no end![15,44]

BIOGRAPHICAL INFORMATION

Robert Root-Bernstein obtained his A.B. in Biochemistry (1975) and his Ph.D. in History of Science (1980) at Princeton University, followed by postdoctoral work at the Salk Institute for Biological Studies, where he was awarded one of the first MacArthur Fellowships (1981). He is currently a Professor of Physiology at Michigan State University where he studies the origin of life, the evolution of physiological control systems, the causes of autoimmune diseases, the nature of scientific creativity, and art–science interactions.

FOOTNOTES

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The authors declare no competing financial interest.

REFERENCES

1 Kuhn, T. S. *Structure of Scientific Revolution*; Chicago University Press: Chicago, 1959.
2 Root-Bernstein, R. *Discovering: Inventing and Solving Problems as the Frontiers of Knowledge*; Harvard University Press: Cambridge, MA, 1989.
3 Crick, F. H. C. Central Dogma of Molecular Biology. *Nature* **1970**, *227*, 561–563.

4 Judson, H. F. *The Eighth Day of Creation: Makers of the Revolution in Biology*; Simon and Schuster: New York, 1979; p 337.

5 Root-Bernstein, R. Amino Acid Pairing. *J. Theor. Biol.* **1982**, *94*, 885–894.

6 Holsworth, D. D.; Kiely, J. S.; Root-Bernstein, R.; Overhiser, R. W. Antisense Peptides: A Comparative Study Focusing on Angiotensin II Antagonists. *Pept. Res.* **1994**, *7*, 185–193.

7 Root-Bernstein, R.; Holsworth, D. D. Antisense Peptides: A Critical Review. *J. Theor. Biol.* **1998**, *190*, 107–119.

8 Siemion, I. Z.; Cebrat, M; Kluczyk, A. The Problem of Amino Acid Complementarity and Antisense Peptides. *Curr. Protein Pept. Sci.* **2004**, *5* (6), 507–527.

9 Biro, J. C. The Proteonomic Code: A Molecular Recognition Code for Proteins. *Theor. Biol. Med. Modell.* **2007**, *4*, 1–45.

10 Mekler, L. B. On the Specific Selective Interaction between Amino Acid Residues of Polypeptide Chains. *Biofizika* **1969**, *14*, 581–584.

11 Blalock, J. E.; Smith, E. M. Hydropathic Anticomplementarity of Amino Acids Based on the Genetic Code. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 203–207.

12 Viza, D. AIDS and Transfer Factor: Myths, Certainties and Realities. *Biotherapy* **1996**, *9*, 17–26.

13 Root-Bernstein, R. On the Origin of the Genetic Code. *J. Theor. Biol.* **1982**, *94*, 895–904.

14 Fernandez, A.; Scheraga, H. A. Insufficiently Dehydrated Hydrogen Bonds as Determinants of Protein Interactions. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (1), 113–118.

15 Csermely, P. *Weak Links. Stabilizers of Complex Systems from Proteins to Social Networks*; Springer: Berlin, 2006.

16 Root-Bernstein, R. Simultaneous Origin of Homochirality, The Genetic Code and Its Directionality. *BioEssays* **2007**, *29* (7), 689–698.

17 Root-Bernstein, R. S. Experimental Test of L- and D-Amino Acid Binding to L- and D-Codons Suggests that Homochirality and Codon Directionality Emerged with the Genetic Code. *Symmetry* **2010**, *2* (2), 1180–120010.3390/sym2021180.

18 Root-Bernstein, R.; Westall, F. C. Serotonin Binding Sites. I. Structures of Sites on Myelin Basic Protein, LHRH, MSH, and ACTH. *Brain Res. Bull.* **1984**, *12*, 425–436.

19 Root-Bernstein, R.; Westall, F. C. Bovine Pineal Antireproductive Tripeptide Binds to Luteinizing Hormone Releasing Hormone: A Model for Peptide Modulation by Sequence Specific Peptide Interaction? *Brain Res. Bull.* **1986**, *7*, 519–528.

20 Root-Bernstein, R. Catecholamines Bind to Enkephalins, Morphiceptin, and Morphine. *Brain Res. Bull.* **1987**, *18* (4), 509–532.

21 Schenk, J. O.; Morocco, M. T.; Ziemba, V. A. Interactions between the Argininyl Moieties of Neurotensin and the Catechol Protons of Dopamine. *J. Neurochem.* **1991**, *57*, 1787–1795.

22 Dillon, P. F.; Root-Bernstein, R.; Sears, P. R.; Olson, L. K. Natural Electrophoresis of Norepinephrine-Ascorbic Acid Complex. *Biophys. J.* **2000**, *79*, 370–376.

23 Root-Bernstein, R.; Dillon, P. F. Small Molecule Complementarity as a Source of Novel Pharmaceutical Agents and Combination Therapies. *Curr. Pharm. Des.* **2008**, *14* (1), 55–62.

24 Doolittle, R. F.; Bork, P. Evolutionarily Mobile Modules in Proteins. *Sci. Am.* **1993**, *269* (4), 50–56.

25 Anzenbacher, P.; Kalous, V. Binding of D-Glucose to Insulin. *Biochim. Biophys. Acta* **1975**, *386*, 603–607.

26 Yu, G. B.; Caspar, D. L. Structure Of Cubic Insulin Crystals in Glucose Solutions. *Biophys. J.* **1998**, *74*, 616–622.

27 Zoete, V.; Meuwly, M.; Karplus, M. Investigation of Glucose Binding Sites on Insulin. *Proteins* **2004**, *55*, 568–581.

28 Root-Bernstein, R.; Dobbelstein, C. Insulin Binds to Glucagon Forming a Complex That Is Hyper-antigenic and Inducing Complementary Antibodies Having an Idiotype-Antiidiotype Relationship. *Autoimmunity* **2001**, *33*, 153–169.

29 Dwyer, D. S. Amino Acid Sequence Homology between Ligands and Their Receptors: Potential Identification of Binding Sites. *Life Sci.* **1989**, *45*, 421–429.

30 Dwyer, D. S. Assembly of Exons from Unitary Transposable Genetic Elements: Implications for the Evolution of Protein-Protein Interactions. *J. Theor. Biol.* **1998**, *194*, 11–27.

31 Root-Bernstein, R. Molecular Complementarity III. Molecular Complementarity As the Basis for Peptide Hormone Evolution. A Bioinformatic Case Study of Insulin, Glucagon, and Gastrin. *J. Theor. Biol.* **2002**, *218*, 71–84.

32 Root-Bernstein, R. Peptide Self-Aggregation and Peptide Complementarity as Bases for the Evolution of Peptide Receptors: A Review. *J. Mol. Recognit.* **2005**, *18*, 40–49.

33 Root-Bernstein, R. S.; Vonck, J. The Insulin Receptor Binds Glucose Altering the Mutual Affinity of Insulin for Its Receptor. *Cell. Mol. Life Sci.* **2009**, *6* (16), 2721–2732.

34 Root-Bernstein, R. S.; Vonck, J. Modularity in Receptor Evolution. Insulin- and Glucagon-Like Peptide Modules as Binding Sites for Insulin and Glucose in the Insulin Receptor. *J. Recept., Ligand Channel Res.* **2010**, *3*, 87–9610.2147/JRLCR.S6737.

35 Root-Bernstein, R. A Modular Insulin-Like Basis for the Evolution of Glucose Transporters (GLUT) with Implications for Diabetes. *Evol. Bioinf.* **2007**, *2*, 317–331.

36 Root-Bernstein, R.; Dillon, P. F. Fostering Adventure Research. A Case Study of the Discovery That Ascorbic Acid Enhances Adrenergic Drug Activity. *Drug Dev. Res.* **2002**, *57*, 58–74.

37 Dillon, P. F.; Root-Bernstein, R. S.; Lieder, C. M. Anti-Oxidant Independent Ascorbate Enhancement of Catecholamine-Induced Contractions of Vascular Smooth Muscle. *Am. J. Physiol., Heart Circ. Physiol.* **2004**, *286*, H2353–H2360.

38 Dillon, P. F.; Root-Bernstein, R.; Lieder, C. M. Ascorbate Enhancement of H1 Histamine Receptor Coincides with Ascorbate Oxidation Inhibition by Histamine Receptors. *Am. J. Physiol., Cell Physiol.* **2006**, *291*, C977–C984.

39 Dillon, P. F.; Root-Bernstein, R.; Robinson, N. E.; Abraham, W. M.; Berney, C. Ascorbate Binding to the Adrenergic Receptor Enhances in Vivo and in Vitro Beta-Adrenergic Sensitivity. *PLoS One* **2010**, *5* (12), No. e15130.

40 Kauffmann, S. A. *The Origins of Order: Self-Organization and Selection in Evolution*; Oxford University Press: Oxford, U.K., 1993.

41 Simon, H. A. *The Sciences of the Artificial*; MIT Press: Cambridge, MA, 1981.

42 Lemke, J. L. Opening Up Closure. Semiotics across Scales. *Ann. N.Y. Acad. Sci.* **2000**, *901*, 100–111.

43 Growney, J. Planning for Interruptions: The Science of Managing Their Effect. *Interfaces* **1981**, *11* (1), 78–83.

44 Root-Bernstein, R.; Dillon, P. F. Molecular Complementarity, I: The Molecular Complementarity Theory of the Origin and Evolution of Life. *J. Theor. Biol.* **1997**, *188*, 447–479.

45 Hunding, A.; Lancet, D.; Kepes, F.; Minsky, A.; Norris, V.; Raine, D.; Sriram, K.; Root-Bernstein, R. Compositional Complementarity and Prebiotic Ecosystems in the Origin of Life. *Bioessays* **2006**, *28* (4), 399–412.

46 Muller-Hill, B. The Function of Auxiliary Operators. *Mol. Microbiol.* **1998**, *29*, 13–18.

47 Root-Bernstein, R. Molecular Complementarity in an Ecosystems-First Theory of the Origin of Life. In *Astrobiology: From Simple Molecules to Primitive Life*; Busiak, V., Navorro-Gonzalez, R., Eds.; American Scientific Publishers: Valencia, California, 2010.

48 Root-Bernstein, R. Natural Selection by a Combination of Ultraviolet Light and Molecular Complementarity Results in Emergent Properties during the Origins of Life. *Life* **2012**, *in press*.

49 Foa, P. P.; Galansino, G. *Glucagon: Chemistry and Function in Health and Disease*; Charles C. Thomas: Springfield, IL, 1962; pp 6, 10, 39, 78.

50 Terasaki, M.; Nomoto, S.; Mita, H.; Shimoyama, A. A Pathway to Aspartic Acid from Urea and Maleic Acid Affected by Ultraviolet Light. *Origins Life Evol. Biospheres* **2002**, *32*, 91–98.

51 Brillouin, L. Negentropy Principle of Information. *J. Appl. Phys.* **1953**, *24* (9), 1152–1163.

52 Mahulikar, S. P.; Herwig, H. Exact Thermodynamic Principles for Dynamic Order Existence and Evolution in Chaos. *Chaos, Solitons Fractals* **2009**, *41* (4), 1939–1948.

53 Weiss, P. A. *Hierarchically Organized Systems in Theory and Practice*; Hafner: New York, 1971.

54 Raine, D. J.; Grondin, Y.; Thellier., M.; Norris, V. Networks as Constrained Thermodynamic Systems. *C. R. Biol.* **2003**, *326* (1), 65–74.

55 Segre, D.; Ben-Eli, D.; Lancet, D. Compositional Genomes: Prebiotic Information Transfer in Mutually Catalytic Noncovalent Assemblies. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4112–4117.

56 Norris., V.; Root-Bernstein, R. The Eukaryotic Cell Originated in the Integration and Redistribution of Hyperstructures from Communities of Prokaryotic Cells Based on Molecular Complementarity. *Int. J. Mol. Sci.* **2009**, *10*, 2611–263210.3390/ijms10062611.

57 Narayanaswamy, R.; Levy, M.; Tsechansky, M.; Stovall, G. M.; O'Conell, J. D.; Mirrielees, J.; Ellington, A. D.; Marcotte, E. M. Widespread Reorganization of Metabolic Enzymes into Reversible Assemblies upon Nutrient Starvation. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106* (25), 10147–10152.

58 Shenhav, B.; Oz, A.; Lancet, D. Coevolution of Compositional Protocells and Their Environment. *Philos. Trans. R. Soc., B* **2007**, *362* (1486), 1813–1819.

59 Shapiro., R. Small Molecule Interactions Were Central to the Origin of Life. *Q. Rev. Biol.* **2006**, *81* (2), 105–125.

60 Root-Bernstein, R. Autoreactive T-Cell Receptor (Vbeta/D/Jbeta) Sequences in Diabetes Are Homologous to Insulin, Glucagon, The Insulin Receptor, and the Glucagon Receptor. *J. Mol. Recognit.* **2009**, *22* (3), 177–187.

61 Root-Bernstein, R . Antigenic Complementarity in the Induction of Autoimmunity: A General Theory and Review. *Autoimmun. Rev.* **2007**, *6* (5), 272–277.