Miller-Urey and Beyond: What Have We Learned About Prebiotic Organic Synthesis Reactions in the Past 60 Years?

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Abstract

The synthesis of amino acids in the Miller-Urey spark-discharge experiments in the early 1950s inspired a strong interest in experimental studies of prebiotic organic chemistry that continues today. Over the years, many of the basic building blocks of life as we know it have been synthesized in the laboratory from simple ingredients, including amino acids, sugars, nucleobases, and membrane-forming lipids. Questions remain, however, concerning whether the conditions that allow synthesis of these compounds in the laboratory accurately simulate those that might have been present on the early Earth, and a closer convergence between plausible prebiotic conditions and laboratory simulations remains a challenge for experimentalists.

INTRODUCTION

The sequence of events leading to the origin of life on Earth persists as one of the greatest unsolved problems in all of science. Yet, although the ultimate solution to the puzzle of life's origin remains obscure, there can be little doubt that considerable progress toward a solution has been made in recent decades. Laboratory experimental investigations of prebiotic chemical reactions have been a major contributor to this progress. Such experiments have, for example, provided constraints on the inventory of organic compounds available on the prebiotic Earth and allowed testing of theories of the early stages of chemical evolution that led to life's origin. The landmark spark-discharge experiments of Stanley Miller and Harold Urey in the early 1950s (Miller 1953, 1955) ushered in the modern era of experimental study of prebiotic chemistry. (Although Miller is listed as the sole author of the initial publications describing these experiments, they are widely referred to as the Miller-Urey experiments to acknowledge the critical role of Miller's doctoral advisor in the conception and design of the research.) This review provides a brief summary of some of the major advances in experimental prebiotic chemistry since the publication of the Miller-Urey experiments six decades ago.

Besides being a popular icon for scientific study of the origin of life, the Miller-Urey experiments stand out as a landmark for a couple of reasons. Experimental chemists certainly explored chemical reactions with prebiotic relevance long before the Miller-Urey experiments. For example, the formose reaction, widely invoked as a major prebiotic pathway for the synthesis of sugars, and the Strecker synthesis of amino acids were both first described in the mid-1800s (Strecker 1850, Butlerow 1861). What distinguished the Miller-Urey experiments from most earlier studies is that they were designed to test a specific hypothesis for the origin of life, and the experiments were conducted under conditions specifically designed to represent those thought to be present on the early Earth (see Bada & Lazcano 2000 and Lazcano & Bada 2003 for interesting historical perspectives on the conceptual design of the experiments and the origin-of-life theories that led up to them). The Miller-Urey experiments also came at a time of steeply increasing knowledge of the structure and function of proteins and nucleic acids, central biochemical components of all known organisms. Notably, the Miller-Urey experiments were first published within months of other papers describing the first amino acid sequence of a protein (Sanger & Thompson 1953) and the double-helix structure of DNA (Watson & Crick 1953). The production in the Miller-Urey experiments of amino acids (which are the basic building blocks of proteins) at conditions that might reasonably represent the early Earth provided a conceptual link between the environment from which life emerged and the types of organic compounds that could plausibly represent the first steps toward life. For perhaps the first time, it became conceivable that the initial stages in the evolution of life could be reproduced in the laboratory. Theories of the origin of life were no longer just matters of speculation; they could be tested by rigorous scientific investigation.

In the years since the publication of the original Miller-Urey experiments, laboratory investigations of prebiotic chemistry and early chemical evolution have become so prolific that it would be impractical to summarize the entire field in a brief overview. Consequently, this review can cover only a small subset of the literature on this topic. In keeping with the legacy of the Miller-Urey experiments, I focus here on laboratory investigations of the prebiotic synthesis of simple organic molecules. Most origin-of-life scenarios require simple organic compounds to be present on the early Earth as building blocks for more complex structures, such as precursors to primordial equivalents of proteins, nucleic acids, and membranes. Accordingly, a prebiotic inventory of simple organic compounds appears to be a necessary prerequisite to initiate chemical evolution on the early Earth. In addition, I include only those experiments that are relevant

to the conditions that might have prevailed on the early Earth. Although delivery of organic matter from external sources including comets, meteorites, and interplanetary dust particles may have contributed significantly to the inventory of prebiotic compounds on the early Earth (Chyba & Sagan 1992), consideration of experimental studies relevant to the formation of organic compounds from these sources would require more extended discussion than is possible here. Nevertheless, some of the diverse organic compounds found within meteorites may have formed on meteorite parent bodies under conditions akin to those on the early Earth, and the results discussed here may be relevant to the formation of those compounds.

Over the years, many different environments have been considered as possible sites for the origin of life. At the time of the Miller-Urey experiments, the most influential prevailing theories proposed that life emerged from a concentrated solution of organic compounds accumulated on the planet's surface (the so-called primordial soup), to which atmospheric and photolytic reactions potentially contributed (Oparin 1938, Haldane 1954). The discovery in the mid-1970s of deep-sea hydrothermal systems and their associated prolific biological communities soon led to proposals that these systems could have been an alternative site for the emergence of life (Corliss et al. 1981, Baross & Hoffman 1985). Since that time, hydrothermal environments have become a primary focus for experimental studies of prebiotic chemistry, in spite of the arguments of Miller and others concerning the unsuitability of such environments for prebiotic reactions (Bernhardt et al. 1984, Miller & Bada 1988). For a brief, visually based overview of some of the current ideas for the origin of life, see **http://exploringorigins.org**.

Thus far, theories of the origin of life can pinpoint neither the specific set of compounds that were involved in the earliest stages of chemical evolution nor what quantities of these compounds were required. In the absence of more definitive constraints, prebiotic organic chemists tend to concentrate, by default, on the chemical components used by extant biological organisms. Because membranes, proteins, and nucleic acids are the main functional components of modern cells, most experimental studies have concentrated on potential pathways for the synthesis of the building blocks of these cellular components. It should be borne in mind, however, that early protometabolic processes may have incorporated a broader suite of organic compounds and only arrived at the present set of components as evolution progressed. Accordingly, some researchers have expanded their studies to include organic compounds not presently used in biology.

Before proceeding, a few things should be kept in mind when considering experimental studies of prebiotic organic chemistry. First, in order to achieve detectable levels of some target compound of interest, prebiotic chemistry experiments are often performed with strongly reactive substrates [e.g., hydrogen cyanide (HCN)] at much higher concentrations than might be expected to occur in prebiotic environments, except under very special circumstances. In addition, the compounds used as reactants in many experiments are so reactive that their spontaneous decomposition to less reactive substrates often competes with the synthetic reaction pathways under study. Furthermore, prebiotic experiments are often performed with a very small number of pure ingredients, precluding many of the competing reactions that would undoubtedly occur in more complex natural systems that contain a diverse mixture of volatiles, dissolved compounds (both organic and inorganic), and mineral surfaces. For these and other reasons, the applicability of many experimental studies to the early Earth has been questioned by some researchers; specific examples of issues raised by these researchers are mentioned throughout the review. Whether or not such criticisms are valid, they are a reminder that prebiotic laboratory simulations should always be evaluated against the backdrop of the conditions that would have been present in environments on the early Earth.
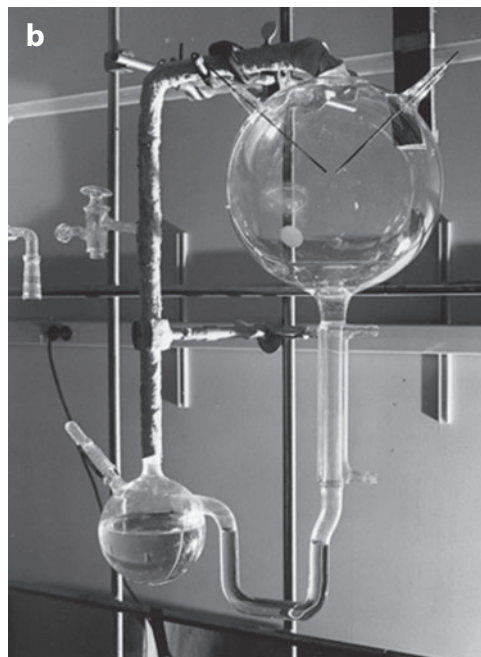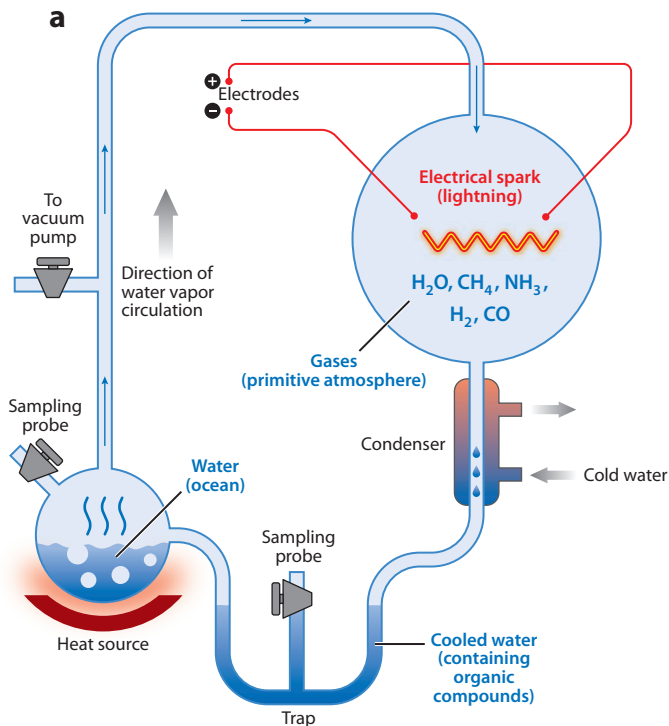
Figure 1

The spark-discharge apparatus used in the Miller-Urey experiments. (*a*) Schematic drawing of the apparatus. (*b*) Photo of the apparatus taken by Stanley Miller (image courtesy of Jeff Bada).

PREBIOTIC SYNTHESIS OF AMINO ACIDS

Proteins play a central role in nearly every biochemical process within modern biological cells. It seems a reasonable presumption, therefore, that primitive proteins participated in the early stages of the development of life and were perhaps even required for the initial steps in chemical evolution to proceed. Accordingly, a considerable number of experimental studies have focused on the prebiotic synthesis of amino acids as building blocks for primitive proteins on the early Earth, and much of the attention paid to the Miller-Urey experiments is attributable to the production of amino acids in those experiments.

The Miller-Urey experiments consisted of two glass flasks—one containing gases nominally representing the primordial atmosphere and the other containing an aqueous solution representing the primordial ocean—connected by tubing, with a pair of electrodes supplying the kind of high-energy input that might be provided by lightning strikes (**Figure 1**) (Miller 1953, 1955; Miller & Urey 1959). During the experiments, spark discharges from the electrodes were passed through the gas while the aqueous solution was boiled. After the experiments ran for a few days, a yellow organic residue accumulated and, when the residue was extracted, five amino acids and several other simple organic compounds were identified as reaction products (**Figure 2**). Recent reanalyses of the products from some of Miller's experiments using modern analytical procedures have found that a much broader range of amino acids was synthesized in the experiments than was originally reported (Johnson et al. 2008, Parker et al. 2011). In addition to amino acids, these experiments
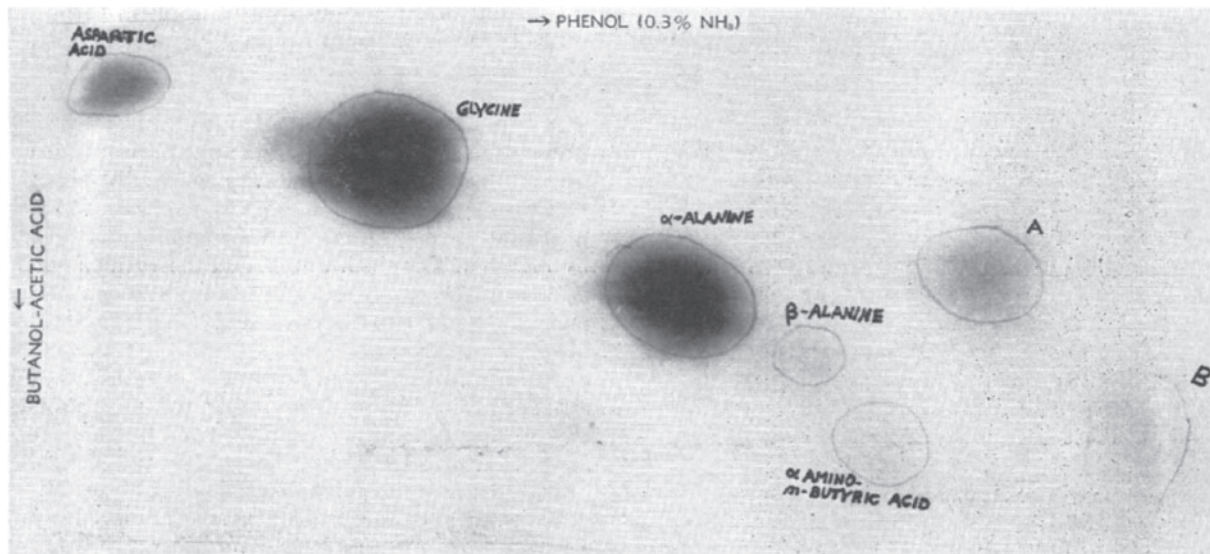
Figure 2

Two-dimensional paper chromatograph used to identify amino acid products from the Miller-Urey experiments. The primary products are glycine and α-alanine. From Miller (1953); reprinted with permission from AAAS.

produce a broad array of other compounds, including urea, HCN, cyanoacetylene, carboxylic acids, and amines.

Although a detailed examination of the reaction pathway for amino acid formation in spark-discharge experiments has never been undertaken, the evidence suggests that the synthesis occurred through a variation of the Strecker synthesis (Miller 1957, Miller & Urey 1959). In the Strecker synthesis, amino acids are produced by reactions among an aldehyde, ammonium, and cyanide to produce an aminonitrile, which then undergoes hydrolysis to form an amino acid (**Figure 3**). A key aspect of the Strecker synthesis with respect to prebiotic chemistry is that it preferentially produces α-amino acids (the form predominantly used by biology) relative to other structural isomers. In the Miller-Urey experiments, it was inferred that aldehydes (including formaldehyde) and HCN formed in the gas phase (the "atmosphere") and then dissolved into the aqueous phase (the "ocean"), where the amino acids were produced via the Strecker synthesis (Miller 1955, 1957; Miller & Urey 1959). If this is the case, the primary role of the gaseous "atmosphere" in the synthesis was as a source of aldehydes and HCN, whereas the actual amino acid synthesis occurred in the "ocean."

α-Amino acids: amino acids in which the amine group (–NH₃) is on the carbon adjacent to the carboxyl group (–COOH) carbon
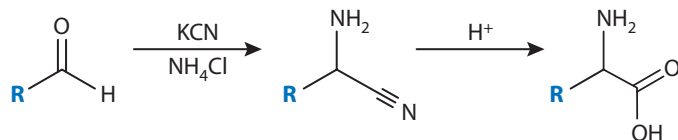

Figure 3

Reaction pathway for formation of α-amino acids by the Strecker synthesis. In the reaction, an aldehyde reacts with ammonium and cyanide [potassium cyanide (KCN) in this case] to form an aminonitrile, which is then hydrolyzed and oxidized to the amino acid. R represents a side group that defines the identity of the amino acid. For R = H, the amino acid is glycine; for R = CH₃, the amino acid is alanine.

The Miller-Urey experiments and subsequent studies using UV light as an energy source demonstrated that, given the right atmospheric composition and sufficiently high energy fluxes, atmospheric processes could lead to the formation of prebiotic amino acids. These compounds could then accumulate in surface environments such as the ocean, lakes, or ponds, where they might contribute to further prebiotic chemical evolution. However, not long after publication of the Miller-Urey experiments, ideas about the composition of the early atmosphere began to change, causing some to question the relevance of the experiments to the early Earth (e.g., Abelson 1966). The gas phase employed in the Miller-Urey experiments was a mixture of highly reduced gases that included methane (CH_4), ammonia (NH_3), and hydrogen (H_2). The composition of the gas phase was based on contemporary models of the early terrestrial atmosphere, which at the time was thought by Urey and others to have been strongly reducing (Bernal 1949, Urey 1952). More recent models of the early atmosphere favor a more neutral composition, intermediate between modern oxidized conditions and the reduced atmosphere favored by Urey and others. These models favor a mildly reduced composition dominated by N_2 and CO_2, with low concentrations of CH_4, H_2, and O_2 (see Kasting 1993).

This changing view of the atmosphere inspired experimentalists to investigate the potential for synthesis of organic compounds in less strongly reduced gas mixtures (e.g., Abelson 1966, Folsome et al. 1981, Schlesinger & Miller 1983, Miyakawa et al. 2002c). In some cases, these experiments produced no detectable amino acids; in others, some amino acids were observed, but with yields that were only a small fraction (<1%) of those reported for strongly reduced atmospheres. More recent results, however, suggest that the yields of amino acids reported in studies with neutral atmospheres may have been underestimated owing to oxidation of the acids during sample processing (Cleaves et al. 2008). Experiments analyzed with oxidation inhibitors added during sample processing resulted in significantly higher amounts of amino acids for CO_2/N_2 gas mixtures than suggested by prior studies, with highest yields obtained when $CaCO_3$ was added to buffer the pH of the aqueous phase (Cleaves et al. 2008). It appears, therefore, that atmospheric processes remain a plausible source of prebiotic amino acids (or of their precursors, aldehydes and HCN) on the early Earth, whatever the oxidation state of the atmosphere.

The high energy inputs provided by spark discharges and UV radiation in Miller-Urey-type experiments soon led other researchers to investigate whether amino acids might be synthesized under milder conditions provided by heating of aqueous solutions of simple carbon and nitrogen sources (Oró et al. 1959, Oró & Kamat 1961, Lowe et al. 1963, Fox & Windsor 1970). Experimental study of the synthesis of amino acids during mild heating of aqueous reactants was reinvigorated in the 1990s as part of an increasing focus on deep-sea hydrothermal systems as a possible site for the origin of life (Hennet et al. 1992, Marshall 1994, Keefe et al. 1995, Huber & Wächtershäuser 2006). In most of these studies, formaldehyde, cyanide [CN^-, in the form of HCN, potassium cyanide (KCN), or sodium cyanide (NaCN)], and ammonium (NH_4^+) are included in some combination as carbon and nitrogen sources. Experiments are conducted by heating aqueous solutions of these compounds at moderate temperatures (typically 80 to 200°C) and then analyzing the solution for production of amino acids (often no other products are identified). In some cases, minerals have been included as potential catalysts.

Experiments using these ingredients have consistently yielded small amounts of amino acids, usually with several different structures represented. In one typical example, Hennet et al. (1992) reacted an aqueous solution containing 0.19 M HCHO, 0.19 M KCN, and 0.23 M NH_4Cl in the presence of iron sulfide and iron oxide minerals and platinum powder at 150°C, for periods up to 54 h. Their experiments yielded millimolar concentrations of the amino acid glycine, along with micromolar concentrations of aspartate, serine, glutamate, alanine, and isoleucine. Other hydrothermal experiments have produced similar results, although there is considerable

variability among experiments in the suite of amino acids produced and their relative abundances. However, substantial differences in ingredients and reaction conditions among the experiments make it difficult to make direct comparisons in order to infer what factors account for the differences in reaction products, and it remains unclear how parameters such as temperature, reactant concentrations, and mineral catalysis impact the synthesis of amino acids in these experiments.

To first order, the amino acid products from hydrothermal experiments are similar to those produced in Miller-Urey-type experiments. Presumably, the formation of amino acids in hydrothermal experiments proceeds by the Strecker synthesis or some closely related process (although the actual reaction mechanism was not determined in any of the studies cited). It is worth noting that, if the reaction pathway inferred by Miller (1957) for spark-discharge experiments is correct, there may be little difference in reaction pathways for amino acid synthesis between the hydrothermal and Miller-Urey-type experiments, except that the ingredients for Strecker synthesis (aldehyde, cyanide, and ammonium) are added by the experimenter in the hydrothermal experiments rather than produced in situ by gas-phase reactions.

A potential alternative source of amino acids in hydrothermal systems could be a variant of the Fischer-Tropsch synthesis. In the Fischer-Tropsch synthesis and related reactions, CO_2 or CO gas reacts with H_2 on the surface of a catalyst to form organic compounds (see McCollom & Seewald 2007 for a review of this process as it relates to geologic environments). Typically, this process produces mainly hydrocarbons, but when ammonia (NH_3) was included along with CO and H_2 gases as reactants with native metal alloys as a catalyst, an assortment of amino acids was observed among the reaction products (Hayatsu et al. 1968, Yoshino et al. 1971). These initial experiments have apparently never been followed up, but it would be interesting to know if the same reactions would occur under hydrothermal conditions or with more common minerals as a catalyst.

Two aspects of the products of prebiotic amino acid synthesis experiments are especially noteworthy. First, the experiments produce an assortment of amino acids with different structures, many of which are not components of proteins in modern organisms. This suggests that the suite of amino acids currently used by biological organisms was selected by evolutionary forces from a broader spectrum of acids available on the early Earth, though how this occurred remains a matter of debate (Cleaves 2010). Second, the amino acids produced in the experiments are racemic mixtures of stereoisomers, with approximately equal proportions of the D and L isomers. In contrast, modern biological organisms almost exclusively use only one stereoisomer of amino acids (the L form) in their proteins. Because it is difficult to imagine how functional compounds with reproducible structures could arise through self-assembly of amino acids from racemic mixtures, some researchers have explored the potential for adsorption on mineral surfaces or other processes to preferentially select and accumulate individual stereoisomers (see Hazen & Sverjensky 2010 and references therein). It remains uncertain, however, how and when during chemical evolution the transition from racemic mixtures to a single isomer occurred.

A factor of persistent concern in all these experimental studies of amino acid synthesis is the degree to which the reaction conditions resemble plausible conditions for the early Earth (see Aubrey et al. 2009 for an extended discussion of this subject). The hydrothermal experiments employ highly reactive compounds that are present at much higher concentrations (0.1 M or greater) than can reasonably be expected to occur in geologic environments. Although the abundance of compounds such as formaldehyde and HCN has apparently never been reported for hydrothermal systems, thermodynamic calculations indicate that they should only be present in trace levels that are orders of magnitude below those employed in experiments, even if hydrothermal systems on the early Earth were more reducing than modern systems (e.g., LaRowe & Regnier 2008). Hydrothermal experiments performed with more realistic concentrations of formaldehyde and cyanide, or with the less reactive forms of carbon and nitrogen that would have predominated

Stereoisomers: compounds with the same molecular composition and sequence of atoms but different spatial orientations of the atoms

on the early Earth, such as CO_2 and N_2, have so far failed to yield detectable levels of amino acids (Keefe et al. 1995, Aubrey et al. 2009). Although atmospheric processes such as those simulated in Miller-Urey-type experiments could be a source supplying HCN and other amino acid precursors to the ocean and hydrothermal systems, the relatively low levels of these compounds produced by atmospheric processes, their reactivity in aqueous solutions, and their relatively low aqueous solubilities combine to argue against attainment of significant concentrations from this source (Aubrey et al. 2009). Attainment of the high concentrations of cyanide, formaldehyde, and other compounds used in laboratory simulations of amino acid synthesis might be possible on the early Earth but might require special environments such as concentration in evaporating ponds or freezing solutions.

Lastly, in evaluating the potential inventory of amino acids available on the early Earth, potential sinks have to be considered as well as sources. Amino acids are relatively unstable, particularly when heated above currently prevailing surface temperatures, and numerous experimental studies have documented their rapid decomposition rates even during mild heating (Vallentyne 1964, 1968; Bada & Miller 1970; Bernhardt et al. 1984; Miller & Bada 1988; Bada et al. 1995; Li & Brill 2003; and many others). Dissolved amino acids exposed to elevated temperatures at the surface of the early Earth or to circulation through hydrothermal systems would undergo decomposition, reducing the inventory of compounds available for prebiotic processes. In hydrothermal systems and elsewhere, reactions to create and destroy amino acids may occur simultaneously, and these compounds may accumulate only in environments where synthesis outpaces decomposition or where adsorption on mineral surfaces and other processes protect the compound from degradation. These considerations notwithstanding, the widespread occurrence of amino acids in chondritic meteorites that have apparently experienced some degree of aqueous alteration on their parent bodies indicates that conditions allowing their accumulation were present in at least some prebiotic geologic environments.

NUCLEOTIDE PRECURSORS TO THE RNA WORLD

The emergence in the late 1980s of the RNA world as a major theory for the origin of life (Gilbert 1986; Joyce 1989, 2002) led to increased attention on the prebiotic synthesis of simple RNA and RNA-like molecules. The RNA in modern organisms is a complex, polymeric structure composed of a variable sequence of four nucleotides, with each individual nucleotide composed of three components: ribose (a 5-carbon sugar), a nucleobase (adenine, cytosine, guanine, or uracil in modern RNA; **Figure 4**), and phosphate. The prebiotic synthesis of simple RNA molecules would therefore require an inventory of ribose and the nucleobases. Assembly of these components into proto-RNA would further require a mechanism to link the ribose and nucleobase together in
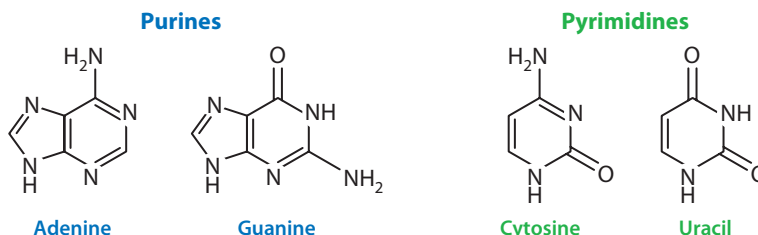

Figure 4

Structures of the nucleobases present in modern RNA, including the purines adenine and guanine and the pyrimidines cytosine and uracil.

the proper configuration to form polymers, and then to activate the combined molecule (called a nucleoside) with a pyrophosphate or some other functional component that would promote formation of a bond between the nucleoside and the growing polymer. As described more fully below, difficulties arising in each of these steps have led to suggestions that RNA may have been preceded by a simpler but structurally similar polymer that only evolved to the current RNA structure over time (e.g., Joyce et al. 1987, Nielsen 1993, Engelhart & Hud 2010). In the interest of brevity, however, only the synthesis of components of modern RNA is discussed here.

Prebiotic Synthesis of Ribose

The most commonly invoked source of sugars in prebiotic environments is the formose reaction, the discovery of which dates back to middle of the nineteenth century (Butlerow 1861). This reaction is essentially a polymerization of formaldehyde, and it proceeds through a series of aldol reactions, reverse aldol reactions, and aldose-ketose isomerizations (Breslow 1959). Glycolaldehyde is the first product of the reaction, and inclusion of this compound as an additional reactant greatly increases the initial reaction rate. In a prebiotic context, experimental studies of sugar synthesis have typically been performed by heating or irradiating aqueous solutions of formaldehyde, and various minerals including calcite and kaolinite have been found to promote the reaction (see, e.g., Gabel & Ponnamperuma 1967, Reid & Orgel 1967, Cairns-Smith et al. 1972). Products typically include a mix of triose, tetrose, pentose, and hexose sugars (sugars with three, four, five, and six carbons, respectively).

Despite widespread references to this process as the primary source of sugars on the early Earth, numerous problems with the formose reaction as a source of ribose in prebiotic environments have become widely recognized (see Shapiro 1988). First, the formose reaction is nondiscriminatory, in the sense that it produces a broad suite of compounds of which ribose represents only a small fraction (~1% or less). The products include sugars with more or fewer carbons, as well as several other structural isomers with the same number of carbons as ribose (Decker et al. 1982). Second, the formose reaction produces potentially useful sugars only if the reaction ceases after a brief reaction time. The sugars are produced as reaction intermediates, and if the reaction is allowed to proceed for longer periods, the products continue to polymerize to form a brown, insoluble product termed tar (see, e.g., Larralde et al. 1995). Experimental studies of prebiotic sugar synthesis have typically avoided this problem by simply running the reaction for only a brief period (a few hours). Third, the formose reaction and related pathways require relatively concentrated solutions to proceed, and it is unclear whether such concentrated solutions could have occurred on the early Earth given the relative instability of formaldehyde (Cleaves 2008).

Recognition of these problems has led experimentalists to explore alternative reaction pathways for ribose synthesis. Perhaps the most promising of these has been inclusion of boron in the reaction mixture, which has been found both to increase yields of pentose sugars (including ribose) among products of the formose reaction and to prevent their degradation during prolonged heating (Prieur 2001, Ricardo et al. 2004). Borate ions in solution are thought to promote preferential synthesis of pentoses by forming complexes with glyceraldehyde, an intermediate in the formose reaction, and to prevent degradation by forming complexes with the resulting sugars. Lead ions have also been shown to enhance yields of pentoses in formose reactions, though not to the same degree as borate (Zubay 1996). Other researchers have found that inclusion of glycolaldehyde phosphate in addition to formaldehyde produces relatively high yields of ribose 2,4-phosphate (Müller et al. 1990), although there is no obvious prebiotic source for the significant amounts of glycolaldehyde phosphate needed to fuel such reactions.

Prebiotic Synthesis of Nucleobases

Owing to their prominent roles in nucleic acids as well as other functional molecules such as adenosine triphosphate (ATP), nucleobases have been the subject of a large number of prebiotic synthesis experiments (**Figure 4**). The first synthesis of adenine in a prebiotic context was reported by Oró (1960) and Oró & Kimball (1961), who incubated highly concentrated ammonium cyanide solutions (1 to 15 M) at temperatures up to 100°C. Following removal of a black polymer and treatment of the supernatant with hydrochloric acid, adenine was identified as a product of the reactions. Yields of adenine in the experiments were low (<1%), and several other reaction products were observed but not identified. Subsequent experiments have shown that guanine is also produced from heating concentrated ammonium cyanide solutions, but at yields 10 to 40 times less than those of adenine (Levy et al. 1999). Adenine and guanine have also been reported in trace amounts from Miller-Urey-type spark-discharge experiments (Yuasa et al. 1984), presumably as a result of polymerization of HCN produced in the gas phase and dissolved into the aqueous solution.

Adenine has a molecular formula of $C_5N_5H_5$, and its structure consists of two rings composed of alternating C and N molecules (**Figure 4**). Consequently, it can be viewed as a condensation product of five molecules of HCN, and its synthesis in concentrated solutions of NH_4CN or HCN would perhaps not be unexpected. Further experimental study (see Sanchez et al. 1966a and references therein) confirmed that adenine synthesis from HCN solutions proceeds through condensation of four HCN molecules to the tetramer diaminomaleonitrile (DAMN), which is then transformed to 4-aminoimidazole-5-carbonitrile (**Figure 5**). Further transformation of the latter produces adenine as well as guanine and other purines (**Figure 5**).

It was soon realized that the highly concentrated solutions employed by Oró and Kimball were unrealistic in the prebiotic world, leading to consideration of whether such reactions would take place in more dilute solutions. Two factors potentially limit the capacity for HCN to be converted to adenine and other purines through the reaction scheme shown in **Figure 5** (Sanchez et al. 1967). First, HCN in aqueous solution is fairly rapidly hydrolyzed to formamide ($HOCNH_2$) and then to formic acid (HCOOH) plus ammonia. Second, although the HCN tetramer DAMN is an important intermediate in purine synthesis, in concentrated solutions this molecule is a transient product that is continually removed through further polymerization with HCN, leading eventually to condensation of intractable high-molecular-weight polymers. The relative contributions of these factors depend on pH and temperature, but at moderate to slightly alkaline pH, the hydrolysis reaction appears to dominate for HCN concentrations lower than approximately 0.01 M (Sanchez et al. 1967), significantly decreasing the potential for purine synthesis by the scheme shown in **Figure 5**.

On the basis of measurements of hydrolysis rates for HCN and formamide, Miyakawa et al. (2002a) estimated that steady-state HCN concentrations in the ocean on the early Earth would have been $<10^{-12}$ M at 100°C and $\sim10^{-6}$ M at 0°C. Such concentrations would appear to preclude synthesis of purines by polymerization of HCN unless some mechanism existed to attain more concentrated solutions. Although hydrothermal systems are another possible environment for purine synthesis, HCN concentrations in hydrothermal fluids are likely to be 10^{-6} M or lower (LaRowe & Regnier 2008).

One proposed mechanism to attain elevated concentrations of HCN in prebiotic environments is the freezing of dilute solutions, and experimental studies of dilute HCN solutions frozen and incubated at -20°C or below have resulted in increased formation of DAMN as well as the synthesis of purines and several amino acids (Sanchez et al. 1966a; Levy et al. 1999, 2000; Miyakawa et al. 2002b). Remarkably, these studies have included analysis of solutions frozen by Stanley Miller in 1972 and incubated for 27 years. One caveat for these studies, however, is that although the experiments were intended to simulate freezing of seawater, the solutions contained none of
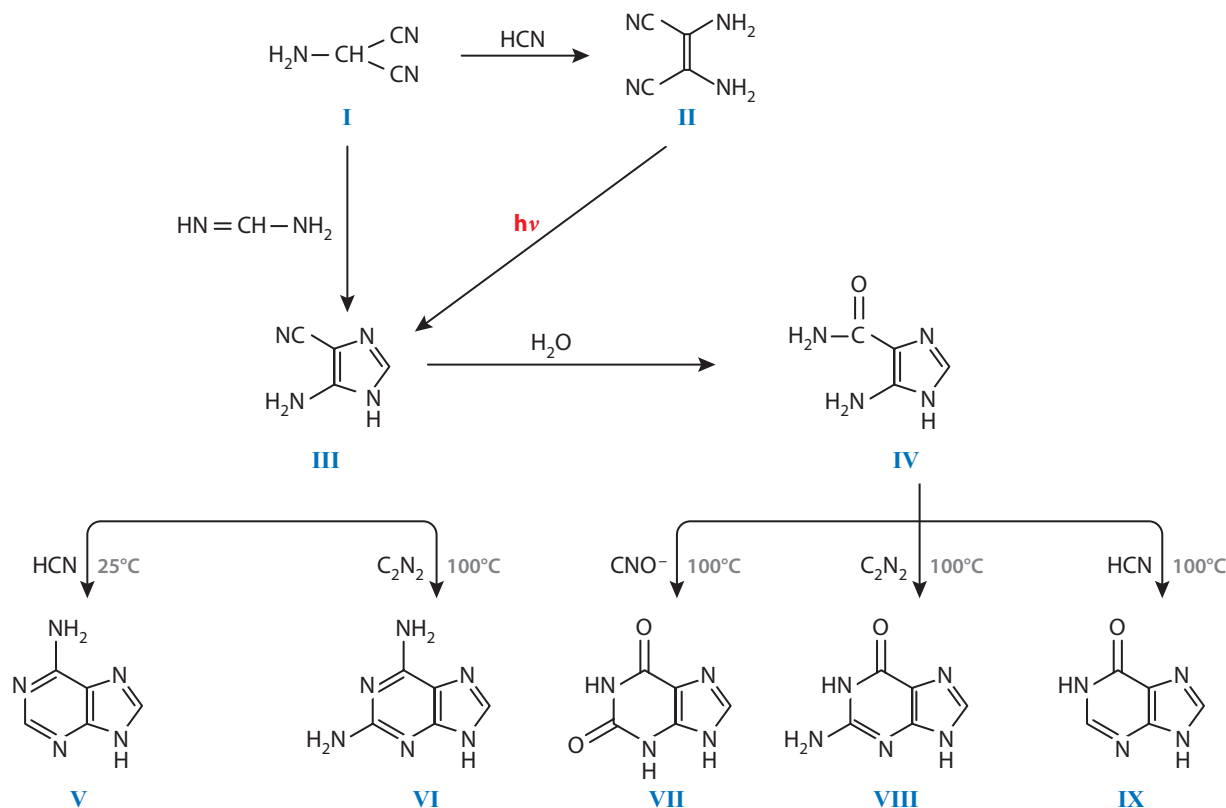
Figure 5

Reaction scheme for the synthesis of purine nucleobases from hydrogen cyanide (HCN). From Sanchez et al. (1966a); reprinted with permission from AAAS.

the salts or other organic compounds that would have been concentrated from seawater along with HCN. The potential impact of these other components on purine synthesis has not been determined.

The structures of cytosine and uracil suggest that some pathway other than HCN polymerization must be involved in their synthesis (**Figure 4**). Early experimental studies reported synthesis of uracil from solutions of malic acid plus urea heated to 100–140°C (Fox & Harada 1961) and synthesis of cytosine from a solution of cyanoacetylene (HCCCN) and potassium cyanate (KOCN) heated to 100°C (Sanchez et al. 1966b). The latter study also showed that cyanoacetylene was among the major products generated in spark-discharge experiments using a mixture of methane and nitrogen gases, suggesting a complete prebiotic pathway for cytosine synthesis. Further study indicated that cytosine yields decreased with concentration of reactants, but 1% yield was still obtained for 2–4 mM concentrations of cyanoacetylene and cyanate (Ferris et al. 1968). That study also showed that hydrolysis of the cytosine produced in the reaction would lead to synthesis of uracil.

As is the case for synthesis of purines from HCN, synthesis of cytosine and uracil from cyanoacetylene and cyanate must compete with decomposition of the reactants. Dissolved cyanate rapidly hydrolyzes to CO_2 plus NH_3, while cyanoacetylene hydrolyzes to cyanoacetaldehyde

(Ferris et al. 1968). The recognition that the instability of cyanate would be an issue in prebiotic environments led Ferris et al. (1974) to explore guanidine and cyanoacetaldehyde as potential ingredients, because they are more stable than cyanate and cyanoacetylene. These authors found that heating these components in water yielded both cytosine and uracil. Synthesis of cytosine and uracil as well as purines has also been reported from heating pure formamide at 160°C in the presence of montmorillonite or TiO_2 (Saladino et al. 2003, 2004). Like cyanate and HCN, however, formamide has a very short lifetime when exposed to water, so it is not clear that experiments performed with pure formamide would have relevance to any prebiotic environment on Earth.

Another alternative to cyanate is urea, but reaction of this compound in dilute solutions with cyanoacetaldehyde failed to yield detectable levels of cytosine (Ferris et al. 1968). Roberston & Miller (1995) therefore proposed that synthesis of cytosine and uracil might have occurred in concentrated solutions of urea and cyanoacetaldehyde formed by evaporation of seawater in isolated lagoons or pools. In support of this possibility, they showed that heating highly concentrated solutions of these components to 100°C resulted in high yields of cytosine and uracil. In a followup study, small amounts of cytosine and uracil formed following evaporation of low-millimolar solutions of urea and cyanoacetaldehyde at 60 to 80°C (Nelson et al. 2001). High yields of uracil along with lesser amounts of cytosine were also produced following incubation of relatively dilute solutions at 0 and −20°C (Cleaves et al. 2006), presumably due to concentration of the reactants by freezing of water. As in the case of synthesis of the purine nucleobases under similar concentration scenarios, these experiments have so far been performed only on pure solutions, and the potential effects of concentrated salts or other compounds on the reactions remain to be determined.

Although nearly all the experimental studies concerning prebiotic formation of nucleobases that have been reported to date have focused on their synthesis from aqueous solutions of simple organic compounds, this is not necessarily the only pathway to their formation on the early Earth. One possible alternative is the Fischer-Tropsch-type (FTT) synthesis; FTT syntheses performed with NH_3 added to CO and H_2 in the reactant gas produce a broad assortment of N-bearing compounds that includes adenine, guanine, uracil, and thymine (Hayatsu et al. 1968, 1972). Synthesis of nucleobases from CO_2, CO, and N_2 is thermodynamically favorable in hydrothermal environments (LaRowe & Regnier 2008), and nucleobases could potentially form by FTT synthesis in such environments. However, the experiments of Hayatsu et al. (1968, 1972) were performed with dry gases using native metals as a catalyst, and it has not yet been determined whether such reactions could proceed under hydrothermal conditions.

As do amino acids and sugars, nucleobases have relatively short lifetimes in aqueous solution. Cytosine is particularly unstable, with a half-life of 19 days at 100°C and 340 days at 25°C (Levy & Miller 1998). Uracil is somewhat more stable, but it still has a half-life of only 12 years at 100°C. For nucleobases to accumulate in prebiotic environments, they must be synthesized at rates that exceed their decomposition. The parameters needed to evaluate synthesis rates for nucleobases in prebiotic environments on the early Earth remain poorly constrained. However, preliminary estimates by Shapiro (1995, 1999) indicate that the balance of production and destruction on a broad scale is unlikely to favor the accumulation of nucleobases, suggesting that their accumulation may be limited to isolated sites where local conditions particularly favor their formation.

Although many advocates of the RNA world appear satisfied that the experimental results outlined above provide sufficient evidence that the basic ingredients to construct nucleosides were generally available on the early Earth, other investigators remain highly skeptical that the reactions described so far truly represent plausible synthetic pathways. A series of papers by Robert Shapiro (1988, 1995, 1999), several of which have already been mentioned here, most succinctly summarized the principal concerns. It would be impractical to include here a comprehensive review of the many arguments both for and against the plausibility of the proposed synthesis reactions on

the early Earth, and the interested reader is referred to the papers by Shapiro for an introduction to the main issues (and to many of the papers referenced therein for opposing views). In any event, the prebiotic plausibility of many proposed pathways for nucleobase and sugar syntheses remains a matter of considerable concern and debate.

Putting It All Together: Synthesis of Nucleosides

Presuming that ribose and nucleobases were formed in the prebiotic environment, the next step toward RNA would have been to link these components together into nucleosides. If the experiments described in previous sections are representative of prebiotic synthesis of nucleobases and sugars, the results hint at some obstacles that must be addressed in any scenario for the prebiotic assembly of nucleosides. The reported syntheses of the individual components of nucleosides have been performed under substantially different chemical conditions, and it is not clear that these conditions are mutually compatible. If not, some process must be invoked to synthesize the parts individually and then to bring them together in a separate environment to form nucleosides. In addition, in most cases the nucleoside components generated in the experiments represent only a minor fraction of a full suite of compounds produced, so that synthesis of a nucleoside would require either that the components be further purified or that some mechanism exist to selectively bring the components together out of a complex mixture. Even if the pure components were brought together, however, the presence of numerous structural isomers for ribose and the nucleobases prevents the facile assembly of nucleosides (Sutherland 2010), and thermodynamics favors the hydrolysis of bonds between ribose and the nucleobases relative to their formation. Taken together, these considerations present a somewhat daunting challenge to envisioning reasonable scenarios for the assemblage of nucleosides on the early Earth, even if the individual steps required for the process can be accomplished in the lab.

Perhaps as a reflection of these difficulties, there have so far been very few reports of successful synthesis of nucleosides directly from ribose and nucleobases, and those that have achieved success have been performed under conditions with questionable applicability to natural environments. For example, Fuller et al. (1972a,b) reported that no nucleosides were formed during heating of aqueous solutions of adenine and ribose, but heating dried mixtures of ribose and a purine produced high yields of the corresponding purine nucleoside. Even with these simple ingredients, however, the products included several structural isomers of the nucleosides rather than just those that were most favorable for the formation of RNA. Whether such reactions would proceed during heating of more complex mixtures of products, like those produced in experiments, is not clear. The first successful synthesis of pyrimidine nucleosides from free bases and unactivated sugars was reported only recently from experiments using the same drying method (Bean et al. 2007).

The many difficulties involved in linking ribose with nucleobases warrants consideration of the possibility that alternative reaction pathways may have been responsible for the formation of nucleosides in prebiotic environments. One such possibility is that the bond between the ribose and the nucleobase is formed at an earlier stage in the synthesis rather than as a final step. This possibility was first explored experimentally by Sanchez & Orgel (1970), who formed the nucleoside cytidine by first reacting ribose with cyanamide (H_2NCH) to form an amino-oxazoline intermediate, then reacting this intermediate with cyanoacetylene to complete the ring of the pyrimidine moiety of the molecule. Hydrolysis of a C–O bond between the ribose and pyrimidine moieties produced α-cytidine with 10–20% yields.

Powner et al. (2009) recently took this idea a step further by proposing a complete synthesis pathway for a pyrimidine nucleotide from a somewhat different set of starting ingredients (**Figure 6**). Rather than starting with ribose, this pathway begins with a reaction of glycolaldehyde
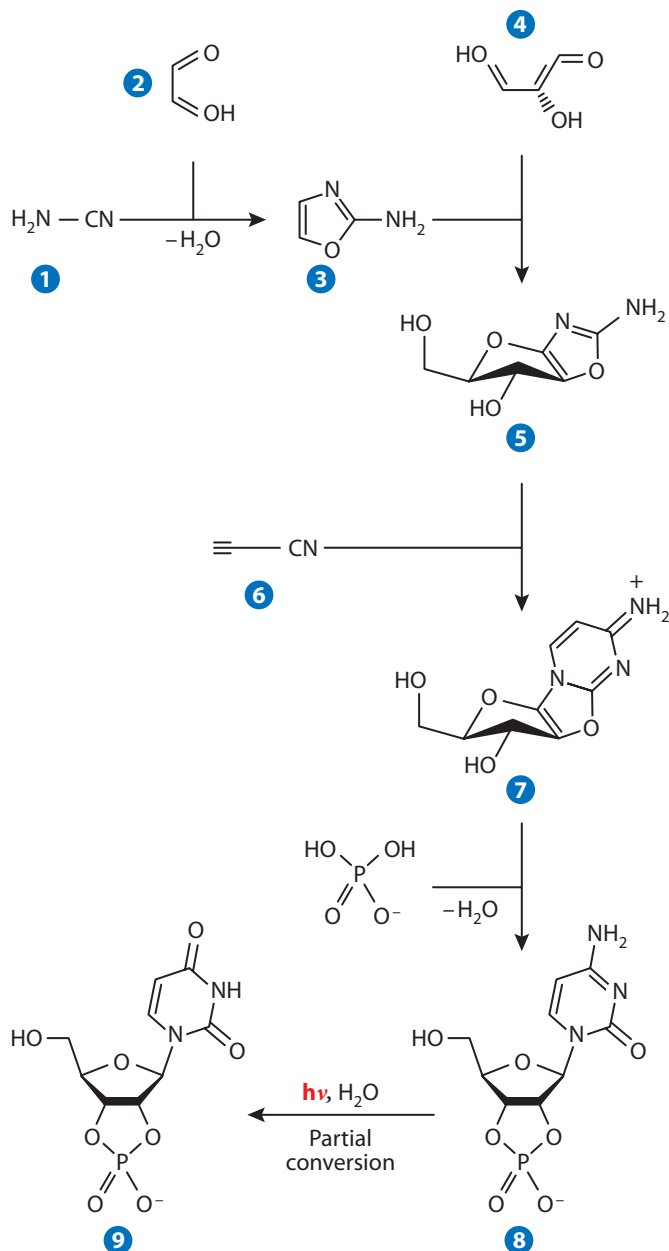
Figure 6

Reaction scheme for the synthesis of pyrimidine nucleotide as described by Powner et al. (2009).

(❷ in **Figure 6**) with cyanamide (❶) to form 2-amino-oxazole (❸), a molecule that includes parts of what will eventually become the ribose and pyrimidine moieties of the nucleotide as well as the bond between them. Reaction of this molecule with glyceraldehyde (❹) produces the same amino-oxazoline intermediate (❺) as in the Sanchez and Orgel scheme. Reaction with cyanoacetylene (❻) produces an anhydrous nucleoside (❼) which, in a phosphate- and urea-bearing solution,

undergoes phosphorylation and hydrolysis to form a β-ribocytidine cyclophosphate (❽). Irradiation with UV light partially converts this product to the corresponding uracil derivative (❾). In a series of experiments, Powner et al. (2009) demonstrated the feasibility of each of these steps and evaluated conditions that would optimize the outcome of each step in the overall synthesis.

Although the experiments described by Powner et al. (2009) are clearly a clever piece of synthetic chemistry, the applicability of such pathways in prebiotic environments still remains far from certain (the title of the paper notwithstanding). All their experimental reactions are conducted with high concentrations of only a few ingredients, with most of the ingredients changing from one step to the next. At several steps in the overall synthesis, the experimenter must intercede to extract and purify the desired product and then expose it to another set of pure reactants under a different set of conditions. Of course, there is no a priori reason to believe that the synthesis of prebiotic organic compounds had to follow a simple pathway, and many compounds could potentially have been exposed to a sequence of different conditions as environments fluctuated (Sutherland 2010). In such multistep scenarios, however, it is difficult to see how desired intermediaries would avoid being dispersed, mixed with other compounds, or exposed to additional reactants that would deflect the intermediaries down other reaction pathways. Nevertheless, the experiments of Sanchez & Orgel (1970) and Powner et al. (2009) point to a potential solution of the problem of nucleotide synthesis, and perhaps further refinements of the synthesis pathway and increasing knowledge of prebiotic environments will allow for clearer convergence between laboratory simulation and geochemical limitations.

MEMBRANE PRECURSORS

Encapsulation of chemical processes within a membrane would have imparted many advantages to evolving chemical systems during the emergence of life (Segre et al. 2001, Szostak et al. 2001, Deamer et al. 2002, Schrum et al. 2010). For instance, encapsulation could concentrate reactants and provide an isolated environment for reaction networks to develop. Encapsulation would also prevent evolving chemical networks from dispersing into the surrounding environments. For these and other reasons, many researchers consider membrane encapsulation at an early stage in prebiotic chemical evolution an essential requirement for the emergence of life.

Conceptually, a membrane may be the most readily formed of the major cellular components on the prebiotic Earth. At high enough concentrations, many amphiphilic organic compounds (i.e., compounds possessing both polar and nonpolar groups) will spontaneously form vesicles consisting of a thin layer of organic material encapsulating an aqueous solution (**Figure 7**). Although many compounds could serve this role, most researchers have focused on fatty acids and fatty alcohols as likely candidates for membrane formation during the emergence of life, for several reasons (Deamer et al. 2002, Schrum et al. 2010, Szostak 2011). Modern biological membranes are composed primarily of phospholipids, but these structures have characteristics such as low permeability that would probably have been disadvantageous in the early days, indicating they probably evolved from simpler forms (Szostak 2011). Fatty acids and alcohols are structurally similar to modern membrane phospholipids and would be logical precursors to those compounds. Fatty acids have also been found in meteorites (Nagy & Bitz 1963, Anders et al. 1973), suggesting that there are plausible prebiotic pathways for their formation in the early Solar System. Furthermore, extracts of meteorites containing these compounds spontaneously form vesicles when hydrated (**Figure 7**) (Deamer et al. 2002).

One potential source of fatty acids in geologic environments on the early Earth would have been the reduction of CO_2 or CO through the Fischer-Tropsch synthesis, or some similar reaction, within hydrothermal environments (McCollom & Seewald 2007). Under most conditions, the
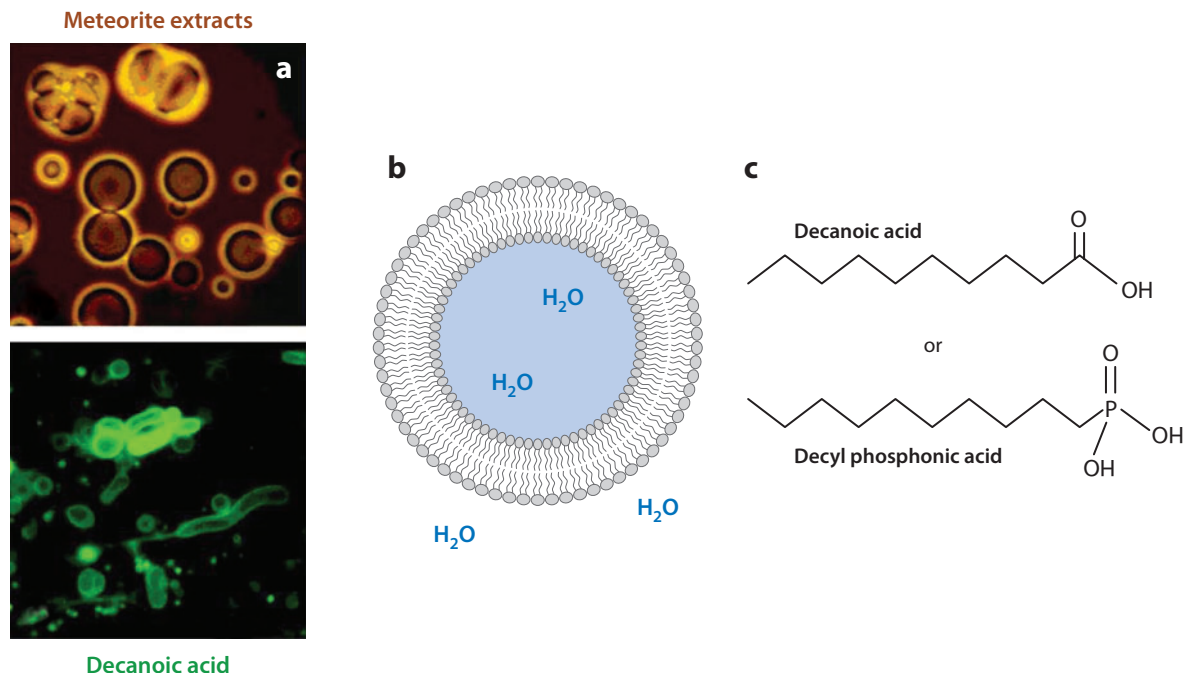
Meteorite extracts

Decanoic acid

Figure 7

Vesicles as possible prebiotic membrane structures. (*a*) Vesicles formed from aqueous suspensions of meteorite extracts (*top*) and decanoic acid (*bottom*). From Deamer et al. (2002); the publisher for this copyrighted material is Mary Ann Liebert, Inc. (*b*) Schematic drawing of vesicle formed from a bilayer of fatty acids. (*c*) Examples of potential membrane-forming aryl compounds, including decanoic acid (*top*) and decyl phosphonic acid (*bottom*).

products of FTT synthesis are dominated by light hydrocarbons, but fatty acids and alcohols are generated as minor by-products in most circumstances. Nooner and colleagues (Nooner et al. 1976, Nooner & Oró 1979) demonstrated experimentally that fatty acids containing up to 16 carbon atoms (C_{16}) were produced during FTT synthesis with metal alloys found in meteorites serving as the catalyst. Subsequent experimental studies have shown that FTT synthesis could form fatty acids and alcohols up to C_{22} under hydrothermal conditions over a temperature range of 100 to 400°C (McCollom et al. 1999, Rushdi & Simoneit 2001, McCollom & Seewald 2006). Fatty acids up to C_{12} have also been identified among the products of Miller-Urey-type experiments (Allen & Ponnamperuma 1967, Yuen et al. 1981), although the mechanism of their formation has not been investigated. Heating of fatty acids with glycerol and urea produces acylglycerols, another potential membrane component (Hargreaves et al. 1977).

The hydrothermal experiments cited above demonstrated that FTT synthesis could proceed under water-saturated conditions. However, the formation of organic compounds in the experiments likely occurred in a gas phase within the reactors, with native iron or steel in the reactor wall serving as the catalyst (see McCollom & Seewald 2007). Numerous other studies have since investigated FTT reactions under conditions intended to more closely simulate those in deep-sea hydrothermal systems, including the use of fluid-rock interactions as the source of H_2, the inclusion of naturally occurring minerals as catalysts, the use of dissolved CO_2 rather than gas as a carbon source, and the exclusion of a gas phase in the reactor (see, e.g., Berndt et al. 1996, Horita & Berndt 1999, McCollom & Seewald 2001, Foustoukos & Seyfried 2004, Fu et al. 2007,

Ji et al. 2008). So far, these studies have produced methane, formic acid, and small amounts of light hydrocarbons as organic products, and none have yielded any evidence of larger molecules that could form membranes. Consequently, a clear demonstration of the formation of membrane-forming compounds at deep-sea hydrothermal conditions remains elusive, although synthesis of these compounds in shallower environments where a gas phase is present appears feasible.

Synthesis by FTT reactions characteristically produces homologous series of linear fatty acids and fatty alcohols that decrease in abundance with increasing number of carbons (see, e.g., McCollom et al. 1999, McCollom & Seewald 2006). Although these compounds are minor products of FTT synthesis, their relatively low volatility and aqueous solubility may allow them to be concentrated during cooling of hydrothermal fluids. Laboratory simulations of prebiotic membrane formation suggest that compounds between 10 and 16 carbons in length and mixtures of fatty acids/fatty alcohols appear to have particularly beneficial properties for early chemical processes (Deamer et al. 2002, Szostak 2011), and FTT synthesis appears well suited to supply such compounds.

Alkylthiols: organic compounds similar to alcohols but containing an –SH rather than an –OH functional group

HYDROTHERMAL SYSTEMS AND EARLY METABOLISM

Partly because of the obstacles involved in the synthesis, assembly, and accumulation of large molecules such as RNA on the early Earth, some scientists favor an alternative route to the origin of life, in which life first emerged from a network of chemical reactions occurring within hydrothermal systems (e.g., Wächtershäuser 1992, 2006; Russell & Hall 2006; Martin et al. 2008). These scenarios are often referred to as chemoautotrophic or metabolism-first theories for the origin of life because the first stages involve the development of core energy and carbon-fixation metabolic pathways, with membranes, proteins, and nucleic acids arising only at a later stage. Submarine hydrothermal systems are particularly favorable environments for these reaction networks to emerge owing to the steep physical and chemical gradients that develop as hydrothermal fluids discharge into seawater, providing both abundant sources of chemical energy to drive reactions and the basic ingredients for chemical reactions, such as CO, H_2S, NH_3, and mineral surfaces.

Hydrothermal systems are typically sulfur-rich environments, and the chemoautotrophic origin theories that are currently most prominent suggest that iron sulfide minerals and organosulfur compounds were primary components of the initial metabolic reaction networks (Wächtershäuser 1992, 2006; Russell & Hall 2006). In an experimental demonstration of this possibility, Huber & Wächtershäuser (1997) showed that acetic acid formed during reaction of aqueous methanethiol (CH_3SH) with CO at 100°C in the presence of metal sulfide minerals. The overall reaction can be expressed as $CH_3SH + CO + H_2O \rightarrow CH_3COOH + H_2S$, and the key step in the reaction is the formation of a C–C bond, which the authors inferred took place by insertion of CO into the C–S bond of methanethiol, catalyzed by the surface of the minerals. The reaction mimics a key step in the energy metabolism pathway of methanogenic archaea, which is catalyzed by an enzyme in which an Fe-S cluster forms the active site.

Within the iron-sulfur world of early hydrothermal environments, molecular hydrogen (H_2) produced by reaction of H_2S with the iron sulfide mineral pyrrhotite (FeS) [$FeS + H_2S \rightarrow FeS_2$ (pyrite) $+ H_2$] could potentially serve as a reductant to reduce CO_2 or CO to prebiotic organic compounds. The capacity for this reaction to produce H_2 was demonstrated experimentally by Drobner et al. (1990) and investigated in much greater detail by Rickard & Luther (1997). Subsequently, Heinen & Lauwers (1996) showed that this pyrite-pulled production of H_2 could indeed lead to synthesis of organic compounds. These authors heated FeS, H_2S, and CO_2 with water in glass vials at temperatures of 25–90°C and observed formation of a homologous series of C_1–C_5 alkylthiols as major products. The alkylthiols decreased in abundance with increasing

number of carbons, and linear butane- and pentanethiol were more abundant than branched forms. More recently, Loison et al. (2010) found that the reactions produced C_1–C_4 carboxylic acids in addition to the thiols. The latter authors also investigated the reaction mechanism and proposed that formation of the alkylthiols and carboxylic acids proceeded through an iterative process involving formation and reduction of thioesters. In this scenario, CO is inserted into the C–S bond of an alkylthiol to form a thiocarboxylic acid with one additional carbon, which is then either converted to a carboxylic acid or reduced to an alkylthiol that can then undergo the same reaction.

The experiments of Heinen & Lauwers (1996) and Loison et al. (2010) suggest the possibility of an iterative process that could generate long-chain alkyl compounds such as fatty acids and fatty alkylthiols through progressive addition of C–C bonds. Although only compounds up to C_5 have been identified in the experiments so far, more extensive reaction may allow development of longer carbon chains. The reaction mechanism appears distinctly different from the Fischer-Tropsch process and would occur under sulfur-rich conditions that are thought to be destructive to Fischer-Tropsch catalysts. Thus, this pathway could have provided an alternative route to formation of membrane-forming compounds, in an environment that might have also been conducive to the development of protometabolic reaction networks.

FUTURE DIRECTIONS

It should be apparent from the foregoing discussions that one of the greatest enduring challenges to understanding the potential synthesis of the basic building blocks of life is to bridge the gap between the conditions that allow reactions to proceed in laboratory experiments and those that were present on the early Earth. Certainly, it is possible to imagine scenarios that would allow the conditions that successfully synthesize prebiotic compounds in laboratory simulations to occur on the early Earth (intermittently drying lagoons, freezing ponds, selective evaporation and wind transport, etc.; see, e.g., Roberston & Miller 1995, Miyakawa et al. 2002a, Sutherland 2010). Such attempts to make the world fit the experiments, however, are never completely satisfying, and a clearer convergence between laboratory simulations and prebiotically plausible conditions is needed.

Progress on this problem must come from several different directions. First, better constraints are needed to define the range of physical and chemical environments that were present on the early Earth; these constraints will come, for example, from examination of the ancient rock record, study of modern analog environments, comparative planetology, and investigation of meteorites, as well as from laboratory and theoretical simulations. Second, laboratory experiments are needed to explore reactions using fluid compositions that more realistically represent those present in natural systems. To optimize the production of target molecules of interest, most prebiotic synthesis experiments have been conducted with a simple suite of reactants, unconstrained by natural environments. Further studies are needed to understand how these reactions are affected by more complex matrices of potential reactants that may either interfere with or promote synthesis reactions. Third, more experiments are needed that use a bottom-up approach, in which the ingredients for synthesis are determined by geochemical reactions rather than imposed by the experimenter (for example, by reacting fluids containing only inorganic ingredients with individual minerals or whole rocks; see, e.g., Heinen & Lauwers 1996, Berndt et al. 1996). Such experiments offer the prospect of more closely simulating geochemical conditions within natural environments than those in which the experimentalist predetermines the starting ingredients.

Solving the puzzle of the origin of life will likely be a prolonged process. However, many pieces are already in place, and with continued progress and effort, the next six decades may well see the solution come into view.

DISCLOSURE STATEMENT

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