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Hydrothermal Chemistry and the Origin of Cellular Life

AU1 ▶

David Deamer,¹ Bruce Damer,¹ and Vladimir Kompanichenko²

Abstract

Two processes required for life's origin are condensation reactions that produce essential biopolymers by a nonenzymatic reaction, and self-assembly of membranous compartments that encapsulate the polymers into populations of protocells. Because life today thrives not just in the temperate ocean and lakes but also in extreme conditions of temperature, salinity, and pH, there is a general assumption that any form of liquid water would be sufficient to support the origin of life as long as there are sources of chemical energy and simple organic compounds. We argue here that the first forms of life would be physically and chemically fragile and would be strongly affected by ionic solutes and pH. A hypothesis emerges from this statement that hot springs associated with volcanic land masses have an ionic composition more conducive to self-assembly and polymerization than seawater. Here we have compared the ionic solutes of seawater with those of terrestrial hot springs. We then describe preliminary experimental results that show how the hypothesis can be tested in a prebiotic analog environment. Key Words: Hydrothermal systems—Ionic composition—Organic solutes—Self-assembly—Polymerization—Ribonucleic acid. *Astrobiology* 19, xxx–xxx.

1. Introduction

A COMMONLY ACCEPTED conclusion related to the origin of life is based on the following logic:

- Life requires liquid water.
- Most of Earth's water is in the ocean.
- Therefore, the origin of life must have occurred in salty seawater.

There is a problem with this line of reasoning, which is that most of the bulk ocean today is a virtual desert in terms of energy sources and nutrients. When hydrothermal vents were first reported (Corliss *et al.*, 1979), it was clear that they could provide energy and potential nutrients, so it was quickly proposed that not only did they support ecosystems based on microbial life but it was conceivable that they could also have been a site for the origin of life (Corliss *et al.*, 1981; Baross and Hoffman, 1985). The first proposals were for so-called black smokers associated with a magma heat source underlying mid-ocean ridges (Russell and Hall, 1997), but Miller and Bada (1988) argued that organic solutes required for life's origin could not withstand the extremely high temperatures encountered by seawater passing through black smokers.

A second version of marine hydrothermal structures was later discovered, which are now referred to as alkaline vents (Kelley *et al.*, 2001). Instead of a magmatic source, the heat

is generated by a chemical reaction called serpentinization in which seawater reacts with peridotite minerals in the seafloor (Kelley *et al.*, 2005). The result is a highly alkaline effluent that interacts with calcium and magnesium in seawater to produce towers of carbonate minerals. The temperature of the fluid is well within the range tolerated by microbial life, and the reaction also produces hydrogen gas, a potential reducing agent. The combination of reducing power and pH gradients across mineral membranes led to proposals that alkaline vents were a plausible site for the origin of life (Martin and Russell, 2003, 2007; Lane and Martin, 2012; Barge *et al.*, 2017).

In general, little attention has been paid to the ionic composition of liquid water in the Hadean and Archean eras and how it might affect processes related to life's origin. However, understanding the interaction of ionic solutes with organic compounds is essential if we are to devise thoughtful experimental approaches. Although this might be considered to be just academic curiosity, the ideas can influence the choice of targets for future life detection missions to Mars, Europa, and Enceladus (Russell *et al.*, 2014; Deamer and Damer, 2015).

To test which site is more conducive for processes leading to the origin of life, we propose the hypothesis that hydrothermal vent fluids and fresh water terrestrial hot springs can be studied experimentally in terms of their ability to provide conditions necessary for life to begin. The processes are not

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strictly chemical, but also include biophysical forces that stabilize self-assembly of organic compounds into membranous compartments. In Section 2, we review temperature, pH, ionic composition, and energy sources available in the seawater circulating through hydrothermal vents, and then compare them with the same set of properties in terrestrial hot springs resembling the hydrothermal fields described by Mulikidjanian *et al.* (2012). Section 3 presents an initial test of the hypothesis in which we attempted to demonstrate polymerization of nucleotides in a New Zealand hot spring. We then discuss how such conditions interact with organic compounds that are solutes and whether they promote or inhibit processes essential for the origin of cellular life.

AU5 ▶ 2. Section 2

2.1. Examples of hydrothermal conditions

To understand aqueous environments likely to be present on the prebiotic Earth and investigate whether they would be conducive to the origin of life, we can learn from prebiotic analog sites that exist today: submarine hydrothermal vents and terrestrial hot springs defined as subaerial hydrothermal fields associated with volcanic land masses. We emphasize that the fresh water in hot springs resembles the water used in most laboratory experiments because it has been distilled from salty seawater and falls on volcanic mineral surfaces as precipitation. The major solutes present are ions derived from basaltic lava and dissolved volcanic gases such as carbon dioxide and sulfur dioxide.

Because the ionic composition of today's seawater is a known quantity, we have chosen it for this comparison. The ionic composition of seawater 4 Ga is less certain, but for the purposes of this review, we assume that the various ion concentrations are at least those of seawater (Fiori *et al.*, 2004), and perhaps even higher salinity. For instance, Knauth (2005) has estimated the salinity of the early ocean to be 1.5 to 2 times greater than seawater today. Marty *et al.* (2018) measured water trapped in Archean quartz and concluded that seawater has approximately the salinity of today's ocean.

2.1.1. Hydrothermal vents. The water circulating through both types of vents begins as seawater but then picks up characteristic solutes as it passes through the underlying mineral matrix. Table 1 shows the composition of seawater, and for comparison Table 2 shows two examples of seawater emitted from black and white smokers. Cationic and anionic equivalents take into account the total positive and negative charges present on monovalent and divalent ions, and should be a nearly exact match reflecting the balance of electrical charges in the solution.

We pay particular attention to the divalent cations and pH of hydrothermal water. The reason is that nucleotides have anionic phosphate as a component, while amino acids and monocarboxylic acids have carboxylate anions in their structure. As we seen in Section 3, these are the groups that form the phosphoester bonds of nucleic acids, the peptide bonds of proteins, as well as the ionized surfaces of lipid membranes. The pH and concentration of divalent cations in an aqueous solution strongly affect the ionic charge on these groups, which in turn alters their ability to engage in polymerization reactions and self-assembly processes.

TABLE 1. IONIC COMPOSITION OF SEAWATER

Seawater pH = 8.1

Na ⁺	469 mM
Cl ⁻	546 mM
Ca ²⁺	10 mM
Mg ²⁺	53 mM
K ⁺	10 mM
HCO ₃ ⁻	2 mM
SO ₄ ²⁻	28 mM
SiO ₂	0
Cationic equivalents	605 mM
Anionic equivalents	604 mM

Adapted from Millero *et al.* (2008).

The lesson from Tables 1 and 2 is that Mg⁺⁺ concentrations in seawater and vent fluids derived from seawater range from 0 to 54 mM, and Ca⁺⁺ concentrations from 10 to 37 mM. Furthermore, the pH range of 3.8 (black smokers) to 9.8 (white smokers) is equivalent to a million-fold difference in hydrogen ion concentration. The pKa's of phosphate, amine, and carboxyl groups fall within this range, so the pH must be taken into account when we consider its effect on chemical reactivity.

Another important physical variable is temperature. The bulk ocean today is near 0–3°C at the seafloor, but the early ocean at the time that life began was likely to have been much warmer because it was still cooling along with Earth's crust from the temperature at which the ocean condensed from the original atmosphere of water vapor. Because of the extreme pressure in the deep ocean, the fluid from black smokers is emitted at temperatures exceeding 300–400°C, well beyond the capacity of microbial life or even dissolved organics to survive. The abundant life associated with black smokers thrives on the surface of the chimneys, which are cooled by seawater, but there is a temperature gradient from the surface into the porous minerals of the chimneys. Life's upper limit of 121°C was measured for microorganisms isolated from the mineral matrix of black smokers (Lovley and Kashefi, 2003). In contrast, the alkaline vent temperatures ranging from 40°C to 70°C are well tolerated by microbial life.

TABLE 2. IONIC COMPOSITION OF HYDROTHERMAL VENT WATER (KELLEY *ET AL.*, 2005)

Lucky Strike (black smoker) pH = 3.8–6.3, T = 308–324°C

Na ⁺	347–426 mM
Cl ⁻	417–472 mM
Ca ²⁺	32–37 mM
Mg ²⁺	0
Cationic equivalents	456 mM (calculated from mean)
Anionic equivalents	444 mM (calculated from mean)

Lost City (white smoker) pH = 9–9.8, T = 40–75°C

Na ⁺	479–485 mM
Cl ⁻	546–549 mM
Ca ²⁺	21–23 mM
Mg ²⁺	9–19 mM
Cationic equivalents	554 mM (calculated from mean)
Anionic equivalents	547 mM (calculated from mean)

AU6 ▶

T1 ▶

T2 ▶

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The upper limit of temperature tolerated by microbial life is set by several biophysical properties of molecular structures that are essential for life processes. One is that genetic information is stored in the double helix of DNA and expressed when ribosomes synthesize proteins from activated amino acids delivered by transfer RNA. The functions of both structures involve making and breaking of hydrogen bonds, and the stability of hydrogen bonds cannot be maintained at temperatures much above 90°C at atmospheric pressure.

A second limitation is that proteins must be precisely folded to maintain catalytic and structural functions. The folding is stabilized by relatively weak forces such as hydrogen bonds and entropic interactions, which again are disrupted when higher temperatures cause unfolding and denaturation. Finally, biological membranes must be able to maintain ion concentration gradients, requiring a continuous nonpolar layer of hydrocarbon chains. The permeability barrier becomes increasingly leaky as temperature increases and at some point the ion transport enzymes embedded in cell membranes are unable to keep up with the leak, leading to dysfunction or even death.

2.1.2. Terrestrial hot springs. Volcanic islands such as Iceland and Hawaii are presumably analogous to similar islands emerging from the ocean at the time of life's origin ~4 billion years ago (van Kranendonk, 2010). They are not caused by tectonic forces associated with the spreading of mid-ocean ridges, but instead result from magma plumes beneath the seafloor of the Atlantic and Pacific oceans. The volcanoes caused by subduction such as those in Kamchatka and the western edge of North and South America are similarly powered by magmatic heat and are supplied with meteoric water distilled from salty seawater. The hydrothermal water of hot springs has been well studied in terms of ionic concentrations, and so, we use them as examples.

There are major differences between the properties of seawater and hot spring water. As noted earlier, hot springs are basically supplied with distilled water in the form of meteoric precipitation, so the ionic concentrations are much lower. Second, the subaerial water circulating in hot springs undergoes continuous cycles of evaporation and hydration. The cycles can be as short as few minutes when water splashing from geyser eruptions falls on hot mineral surfaces. Longer cycles occur when water levels in pools are caused to fluctuate by variable influx related to hot spring activity, leaving a "bathtub ring" of dry organic films on the rocks that contain the pools. The longest cycles measured in weeks and years are related to periods of precipitation followed by dry intervals. The main point is that during evaporation, the films accumulating on mineral surfaces contain compounds that cannot react in dilute solutions, but readily do so in extremely concentrated films.

We now describe the ionic species in hot spring water and then compare them to the ionic composition of seawater and hydrothermal vents.

2.1.3. Terrestrial hot springs in the Kamchatka Peninsula. The Kamchatka Peninsula represents a useful analogue of the prebiotic environment because it contains a variety of hydrothermal discharges distributed over a vast region. The best-explored sites are the hydrothermal systems associated

TABLE 3. IONIC COMPOSITION OF KAMCHATKA HOT SPRING WATER SUMMARIZED FROM FOUR SAMPLES

<i>Ion</i>	<i>Range (mM)</i>
Na ⁺	0.61–48
K ⁺	0.12–1.56
Ca ²⁺	0.1–2.5
Mg ²⁺	(μM)
SO ₄ ²⁻	1.2–4.8
Cl ⁻	0.04–41
Total ionic equivalents	18.8 mM (mean value of four samples)

with Mount Mutnovsky, an active volcano, and the Uzon Caldera. They contain multiple hydrothermal sites consisting of hot pools and lakes, boiling springs and several geysers. The intense hydrothermal processing in the fields is maintained by the underlying magma and volcanic activity.

The physical and chemical composition of water in the Mutnovsky and Uzon systems is remarkably diverse [see Table 3, adapted from Kompanichenko (2017)], with temperatures ranging from 50–60°C to 95–100°C and pH 2–10. Salinity varies from a dilute salt content of ~0.5 g/L to brackish water having 4–4.5 g/L. Specific concentrations of monovalent cations are 0.3–60 mM Na⁺, 0.01–3.6 mM K⁺, and <0.1 to 1 mM NH₄⁺. Divalent cations are usually within the intervals of 0.1–2.5 mM Ca²⁺ and <0.2 mM Mg²⁺ in clear water pools. The main anions are chloride from 0.04 to 41 mM, sulfate from 1.2 to 4.8 mM, and carbonate both as CO₃²⁻ and HCO₃⁻ <0.1–10 mM depending on pH.

The Kamchatka results can be compared with another hydrothermal site, that of Yellowstone National Park (Table 4). The ionic compositions are similar, even though the Kamchatka hot springs are associated with recent eruptions, while the last Yellowstone eruption occurred more than 600,000 years ago. This suggests that ionic solutes dissolving from minerals quickly reach concentrations that are maintained in a relatively dilute steady state because the water is continuously being turned over by precipitation and outflow.

The ion concentrations in Yellowstone hydrothermal water range from 0.01 to 13 mM, and pH from 3.3 to 7.9. Significantly, the divalent cation concentrations are micromolar to 7 mM. This is in contrast to the divalent cations in seawater (10 mM Ca²⁺ and 53 mM Mg²⁺) and alkaline hydrothermal vents (21–23 mM Ca²⁺ and 9–19 mM Mg²⁺). To illustrate the striking differences in the ionic composition of seawater and hot spring water, the two are compared in Fig. 1.

By comparing ionic compositions of Kamchatka and Yellowstone hot spring water with the same solutes in submarine hydrothermal vents and seawater, we can draw some important conclusions. First, the anionic/cationic equivalents in the samples from the hydrothermal subaerial systems range from ~1 to 48 mM, but even the highest of these values is an order of magnitude less than the total ionic equivalents of submarine vents (450–550 mM) and seawater (600 mM). Because the water in hot springs is distilled from seawater by evaporation and then falls on volcanic land masses as precipitation, only small amounts of mono- and divalent ions are dissolved from the minerals in contact with the water.

◀AU17

◀T3

◀T4

◀F1

AU18 ▶ TABLE 4. IONIC COMPOSITION FROM TWO SAMPLES OF YELLOWSTONE HOT SPRING WATER (MILSHTEYN *ET AL.*, 2018)

<i>“Bison Pool” outflow, lower geyser basin</i>		
AU19 ▶ Temperature	68.4°C	
pH	7.9	
Na	12.52 mM	
Ca	0.01 mM	
Mg	0.08 μM	
K	0.36 mM	
DIC (HCO ₃ [−])	5.42 mM	
Cl	6.12 mM	
F	1.21 mM	
SO ₄	0.17 mM	
SiO ₂ (dissolved)	3.60 mM	
Cationic equivalents	12.90 mM	
Anionic equivalents	13.09 mM	
<i>Midway geyser basin</i>		
Temperature	53.0°C	
pH	3.3	
Na	0.39 mM	
Ca	0.02 mM	
Mg	0.01 mM	
K	0.17 mM	
DIC (H ₂ CO ₃)	0.17 mM	
Cl	0.14 mM	
F	0.03 mM	
SO ₄	0.32 mM	
SiO ₂ (dissolved)	Not measured	
Cationic equivalents	1.12 mM	
Anionic equivalents	0.67 mM	
<i>Mammoth hot springs</i>		
<i>T</i> = 71.5°C ± 2°C (<i>n</i> = 7)		<i>Mean</i>
pH	6.5 ± 0.3 (<i>n</i> = 6)	6.5
Na	131 ± 7 ppm (<i>n</i> = 8)	5.7 mM
Cl	166 ± 3 ppm (<i>n</i> = 9)	4.6 mM
Ca	305 ± 60 ppm (<i>n</i> = 6)	7 mM
Mg	68 ± 5.7 ppm (<i>n</i> = 7)	3 mM
K	55 ± 8 ppm (<i>n</i> = 7)	1.4 mM
HCO ₃	725 ± 135 ppm (<i>n</i> = 5)	12 mM
SO ₄	510 ± 38 ppm (<i>n</i> = 7)	5 mM
SiO ₂	53 ± 3.8 ppm (<i>n</i> = 8)	0.9 mM
Cationic equivalents		27 mM
Anionic equivalents		26.6 mM
<i>Norris geyser basin</i>		
<i>T</i> = 86°C ± 6°C (<i>n</i> = 20)		<i>Mean</i>
pH	4.1 ± 0.7 (<i>n</i> = 14)	4.1
Na	299 ± 130 ppm (<i>n</i> = 18)	13 mM
Cl	611 ± 171 ppm (<i>n</i> = 10)	17 mM
Ca	5.9 ± 3.8 ppm (<i>n</i> = 16)	0.120 mM
Mg	0.6 ± 0.3 ppm (<i>n</i> = 16)	0.026 mM
K	53 ± 26 ppm (<i>n</i> = 38)	1.4 mM
H ₂ CO ₃		~0
SO ₄	131 ± 68 ppm (<i>n</i> = 4)	1.3 mM
SiO ₂	450 ± 123 ppm (<i>n</i> = 9)	7.5 mM
Cationic equivalents		14.5 mM
Anionic equivalents		18.3 mM

AU20 ▶ Ionic composition of Yellowstone hot spring water reported by Rowe *et al.* (1973). Concentrations are shown both as ppm and calculated as millimolar (mM) from the mean.

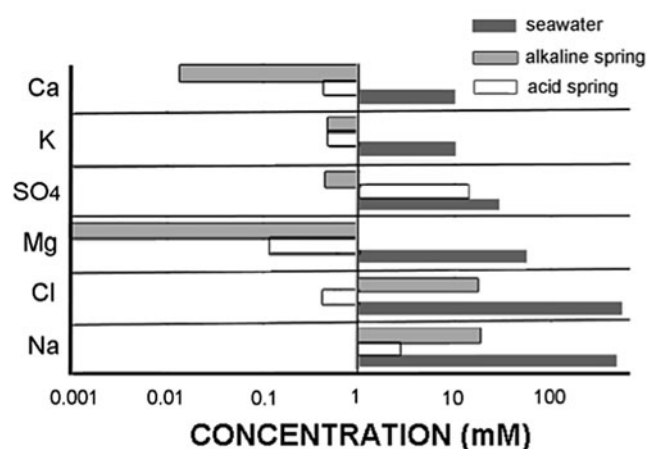


FIG. 1. Ionic composition of seawater compared with hydrothermal water from an alkaline (pH 8.1) and acidic (pH 1.8) hot spring in the Taupo Volcanic Zone near Rotorua, New Zealand (Table 8). Note that the concentrations are presented on a logarithmic scale. The sodium chloride concentration of seawater is ~25 times that of hot spring water, and the differences in divalent cation concentrations (magnesium and calcium) can be even greater.

Terrestrial hot springs also differ from submarine vents by having a remarkable diversity and distribution of properties. For instance, the Uzon hydrothermal system is located above a magmatic chamber 2–3 km beneath the surface. An area 100 m in diameter can have discharges of strongly acidic to neutral pH water containing mostly sodium chloride, or alkaline and slightly acidic water with sodium sulfate and carbonate as the main ionic species (Karpov, 1992). Finally, the water in volcanic hydrothermal sites undergoes continuous cycles of evaporation and re-filling. As discussed later, concentration of organic solutes during wet/dry cycles provides a source of chemical energy that can drive condensation reactions related to synthesis of biopolymers.

Such diversity in hot springs is not surprising, considering the complex interactions between solid mineral surfaces, hot water, and the atmosphere. The geochemical and structural heterogeneity of fresh water hydrothermal systems would also provide highly diverse environments on the early Earth, some of which would be able to concentrate organic solutes and drive their transformation into more complex systems with functions that can be incorporated into primitive forms of life.

2.2. Organic solutes in Kamchatka hydrothermal systems

An important question concerns whether there would have been sufficient concentrations of organic compounds on the prebiotic Earth that could assemble into molecular systems capable of evolving into primitive forms of life. Two possible sources have been proposed: delivery of extraterrestrial material as interplanetary dust particles, comets and meteorites, or geochemical synthesis in the Earth's crust and atmosphere (Chyba and Sagan, 1993; Ehrenfreund and Charnley, 2000; Hazen *et al.*, 2002; Benner *et al.*, 2010). It is uncertain which was the primary source, but delivery of

◀ **AU7**

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TABLE 5. CONCENTRATION OF SOLUBLE, COLLOIDAL, AND TOTAL ORGANIC CARBON IN KULDUR HYDROTHERMAL SYSTEM

Sampling sites	Content, mg C/L		
	$C_{sol.}$	$C_{col.}$	$C_{tot.}$
Well 1–87 (T=72°C)	0.4	0.04	0.4
Well 2–87 (T=71°C)	0.3	0	0.3
Well 3–87 (T=60°C)	3.2	0.5	3.7
Well 3–51 (T=55°C)	2.2	0.03	2.2
Well 5–51 (T=22°C)	1	0.02	1

Analyses were performed by S.I. Levshina at the Institute of Water and Ecological Problems FEB RAS (Poturay, 2013; Kompanichenko *et al.*, 2015).

$C_{col.}$ = carbon organic colloidal; $C_{sol.}$ = carbon organic soluble; $C_{tot.}$ = total carbon organic.

organic compounds on meteorites is still happening today so there is little doubt that this process was occurring at much greater rates 4 billion years ago before life began. Because no molecular oxygen was present in the atmosphere, organic compounds would have survived for long periods of time.

If the organic compounds fell into the ocean, they would dissolve into an extremely dilute solution that could not support chemical reactions, but those falling on volcanic land masses would accumulate and become concentrated in hydrothermal pools. The rate at which accumulation and turnover of organic solutes would occur was explored in a computational model by Pearce *et al.* (2017), who used adenine as an example of how an important nucleobase delivered by meteoritic infall would be processed in a hydrothermal environment.

An interesting question is whether organic compounds might be circulating in fresh water terrestrial hot springs today. Because organic compounds associated with life have

dominated Earth's surface for half a billion years, organic solutes in hydrothermal water are likely to have had a biological rather than an abiotic source, but if they are present in hydrothermal water, such solutes can provide a model system for the way prebiotic organic carbon might have accumulated and been processed in hot spring water.

Organic compounds have in fact been detected in the water of Mutnovsky and Uzon hydrothermal systems. Table 5 shows analyses of five samples that were taken from drilled wells to avoid surface contamination. The concentrations are very dilute, ranging from 1 to 3 mg total organic carbon per liter of water. Significantly, the solutes are hydrocarbons and monocarboxylic acids rather than metabolites such as amino acids and carbohydrates. This is expected, because hydrocarbon derivatives are by far the most stable compounds associated with life (Georgiou and Deamer, 2016). For instance, petroleum is derived from microbial populations of phytoplankton and zooplankton that thrived in shallow seas over a hundred million years ago. Only the hydrocarbons and their derivatives produced by pressure and elevated temperatures remain.

Figure 2 shows a typical chromatogram of alkanes in a water sample from a drilled well. The main peaks are 9–11 carbons in length, followed by a series of alkanes up to 26 carbons long. Given a biological source of such hydrocarbons, the alkanes are probably derived from fatty acids synthesized by microbial life and buried in sediments. Tectonic subduction then exposes the sediments to volcanic temperature and pressure regimens that cause cracking, decarboxylation, and pyrolysis reactions that produce alkanes. Such reactions are familiar and well studied because they also form petroleum hydrocarbons from biological material buried in sediments and mined as fossil fuels.

Detailed analytical results of organic compounds in hydrothermal water have been described in recent publications (Kompanichenko *et al.*, 2015, 2016; Kompanichenko,

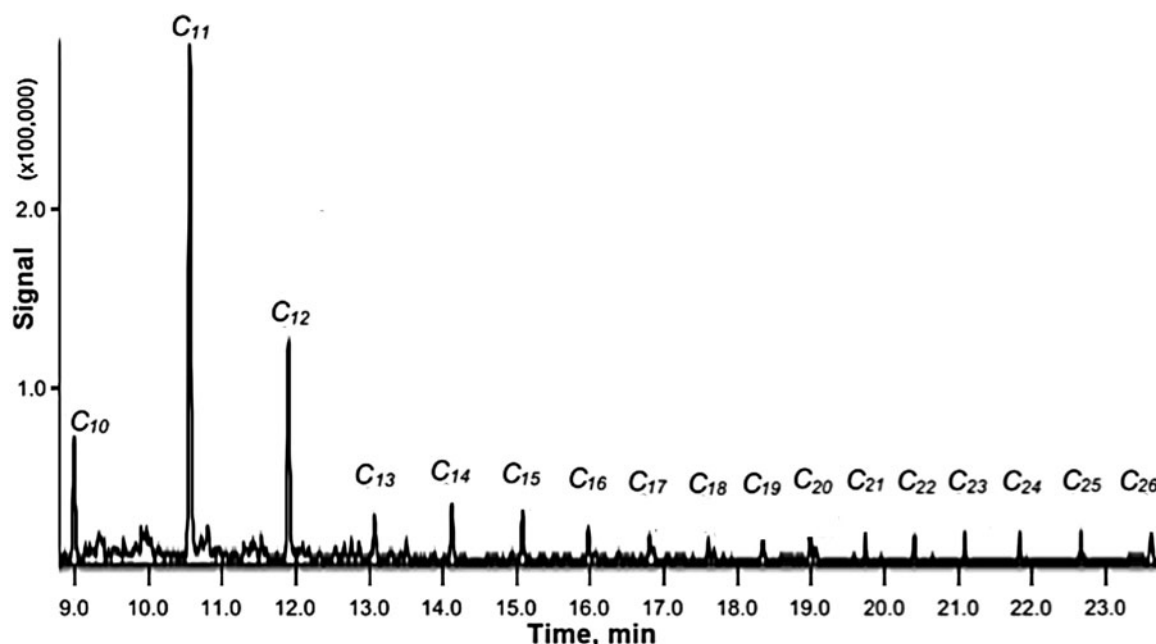


FIG. 2. Chromatogram of n-alkanes in the condensate of water/vapor mixture from well 4-E.

TABLE 6. HYDROCARBONS AND LIPID PRECURSORS DETECTED IN HYDROTHERMAL SYSTEMS OF KAMCHATKA AND NEARBY AREAS (KOMPANICHENKO *ET AL.*, 2016)

Class	Homologous series	No. of compounds in series
Hydrocarbons	Normal alkanes	29
	Isoalkanes	33
	Isoprenes	2
	Alkenes	12
	Isoalkenes	2
	Halogenated hydrocarbons	6
Lipid precursors	Alcohols	8
	Aldehydes	6
	Ketones	4
	Carboxylic acids	20
	Esters	28
	Terpenes	4
	Monoglycerides	1
Total	13	155

2017). In total, 243 organic compounds of moderate and high volatility that belong to 24 homologous series have been detected in the Kamchatka Peninsula. A summary list of hydrocarbons and lipid precursors analyzed in hydrothermal systems is given in Table 6. The most important lipid precursors are carboxylic acids that were detected in approximately half of the samples, including hexanoic, octanoic, nonanoic, decanoic, dodecanoic, tridecanoic, and tetradecanoic acid. Carboxylic acids can assemble into membranous vesicles if they have nine or more carbons in their hydrocarbon chains (Apel *et al.*, 2002) and are the essential hydrophobic groups of phospholipids composing biological membranes. Because they are present in carbonaceous meteorites, it seems probable that carboxylic acids were also incorporated into primitive cell membranes.

Because of the biological significance of long-chain monocarboxylic acids, we pay particular attention to their presence in hydrothermal water. The relative content of carboxylic acids in the samples from Mutnovsky and Uzon thermal fields ranges from 0% to 43.4% of the total peak area (Table 7). The relative concentration of carboxylic acids to alkanes in all samples ranges from 20% to 45%.

Samples with higher concentrations of carboxylic acids also have lower concentrations of normal alkanes and isoalkanes. Thus, the highest content of carboxylic acids in sample 3 (43.4%) and 9 (18.9%) corresponds to the lowest content of normal alkanes and isoalkanes (3.1% and 9.4%). This can be explained by a continuous recombination of organic molecules in the hydrothermal systems, where significant fluctuations of pressure, temperature, pH, and Eh commonly occur.

The most likely source of organic compounds from Mutnovsky and Uzon hydrothermal systems is pyrolysis of residual material derived from plant tissue or microorganisms (Kompanichenko *et al.*, 2016). Can an abiotic source be excluded? Hydrocarbon chains synthesized by a nonbiological process will have equal numbers of odd and even numbers of carbon in the chains, so that the odd/even ratio is 1:1. However, biological fatty acids are synthesized metabolically from acetyl coenzyme A. Each step of the synthesis adds two carbons to a growing chain so that the hydrocarbon chain lengths will be even numbers that vary in steps of two carbons. When fatty acids are buried in sediments followed by exposure to high temperatures and pressures, decarboxylation begins to produce increasing amounts of chains with odd numbers of carbon atoms, which means that a ratio either less than or greater than 1 is a biomarker for fatty acids synthesized by metabolism rather than an abiotic process such as the Fischer–Tropsch reaction (Georgiou and Deamer, 2014). Less than 1 indicates that less than half of the originally pure even numbered chains have become decarboxylated, while more than 1 means that more than half of the chains have lost a carboxyl group.

In water samples from most hot pools, the odd/even ratio is 1.3–1.5, which implies a biological source of the hydrocarbons. However, in a few samples, the odd/even ratio is 0.9–1.2, but this does not imply an abiotic source. A more likely explanation is that these alkanes have undergone more extensive geothermal processing that includes chain scission (cracking), which tends to randomize chain lengths. Similar trends in the odd/even ratio are seen in oil shales from Oklahoma in the United States (Georgiou and Deamer, 2014). The presence of halogenated hydrocarbons in the Uzon system (Table 6) is also accounted for by geothermal processing of hydrocarbons because microbial populations do not produce such compounds.

TABLE 7. RELATIVE COMPOSITION OF ALKANES AND CARBOXYLIC ACIDS IN THE MUTNOVSKY AND UZON HYDROTHERMAL SYSTEMS (KOMPANICHENKO *ET AL.*, 2016)

Homologous series	Area of peaks, % (in brackets—no. of compounds in series)								
	Sampling sites								
	1	2	3	4	5	6	7	8	9
Normal alkanes	16.7 (17)	31.8 (7)	2.9 (8)	32.8 (8)	19.4 (6)	32.6 (14)	19.9 (5)	41.8 (9)	7.1 (15)
Isoalkanes	7 (4)	0.7 (1)	0.2 (1)	6.9 (8)	—	—	—	3.8 (2)	2.3 (9)
Carboxylic acids	—	—	43.4 (12)	4.1 (1)	—	3.4 (1)	—	—	18.9 (7)

Compounds not appearing in the table are absent in FIC and SIM chromatograms.

Sampling sites. Mutnovsky system: 1—condensate of water/steam mixture from well No. 4-E, T=175°C, pH=4 (Dachnoye field); 2—water pool 1, T=95°C, pH=3.5 (Dachnoye field); 3—water pool 2, T=90°C, pH=3 (Dachnoye field); 4—water pool 1, T>90°C (Donnoye field); 5—water-mud pool 2, T>90°C, pH=2.2 (Donnoye field); 6—condensate of water/steam mixture from well No. 3, T=95°C, pH=9 (Northern Mutnovsky field). Uzon system: 7—water-mud pool Burlyaschiy, T=84°C, pH=2.6; 8—clear water from short well K-4, T=99°C, pH=6.8; 9—geyser Shaman, T=100°C, pH=5.85.

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In conclusion, the results are consistent with a process involving continuous transformation of organic material in hydrothermal systems, in which various physical and chemical processes affect the final distribution of products. Similar processes would presumably occur on early Earth. Organic compounds either delivered as extraterrestrial infall or synthesized by geochemical reactions would circulate in hydrothermal systems and undergo chemical evolution into increasingly stable compounds such as alkanes and fatty acids. It is important to note that volcanic vapors are near 100°C, a temperature at which shorter chain hydrocarbon derivatives evaporate. For instance, because of evaporation, hydrocarbons and their derivatives up to decane and decanoic acid would not be present for very long in hydrothermal pools at near-boiling temperatures. On the contrary, Mansy and Szostak (2008) have demonstrated that vesicles composed of longer chain fatty acids are stable at near-boiling temperatures, so a significant question to be addressed in the future research is to establish mixtures of plausible prebiotic amphiphiles that can form stable membranes at elevated temperatures.

3. Section 3

Given the physical and chemical properties of seawater circulating through alkaline hydrothermal vents and fresh water circulating in terrestrial hot springs associated with volcanic land masses, we now describe how those properties affect processes related to the origin of life: self-assembly, concentration of reactants, and polymerization reactions.

3.1. Self-assembly in hydrothermal water

The ability of amphiphilic compounds to spontaneously assemble into membranous compartments is obviously an essential step in the evolutionary pathway to the origin of cellular life. All living cells today have boundaries of lipid membranes, most often as a bilayer composed of phospholipids synthesized by enzyme-catalyzed metabolism. In the absence of metabolism, complex phospholipids were unlikely to be available on the prebiotic Earth, but it is virtually certain that other amphiphiles were present.

The simplest amphiphilic compounds capable of self-assembly into membranous compartments are monocarboxylic acids referred to as fatty acids in biochemical terminology. Typical fatty acids have a hydrocarbon chain ranging from 10 to 18 carbons in length with a carboxyl group at one end. The length of chain determines the temperature at which it undergoes a gel to fluid phase transition. For instance, the phase transition temperature (T_m) of 12 carbon dodecanoic acid (lauric acid) is 44°C, while T_m of hexadecanoic acid (palmitic acid) with 16 carbons is 64°C. Depending on pH, the carboxyl group can be either protonated and neutral (-COOH) or anionic (-COO⁻), which can strongly affect the physical properties. For instance, protonated dodecanoic acid above its T_m forms oily droplets at acidic pH ranges and micelles at alkaline pH ranges, but at a pH near its pK_a , it assembles into membranous vesicles. Figure 3 shows a molecular dynamics simulation of a fatty acid bilayer, which composes membranous vesicles emerging from a droplet of dodecanoic acid in a hydrothermal water sample taken from a Yellowstone hot spring (Milshteyn *et al.*, 2018).

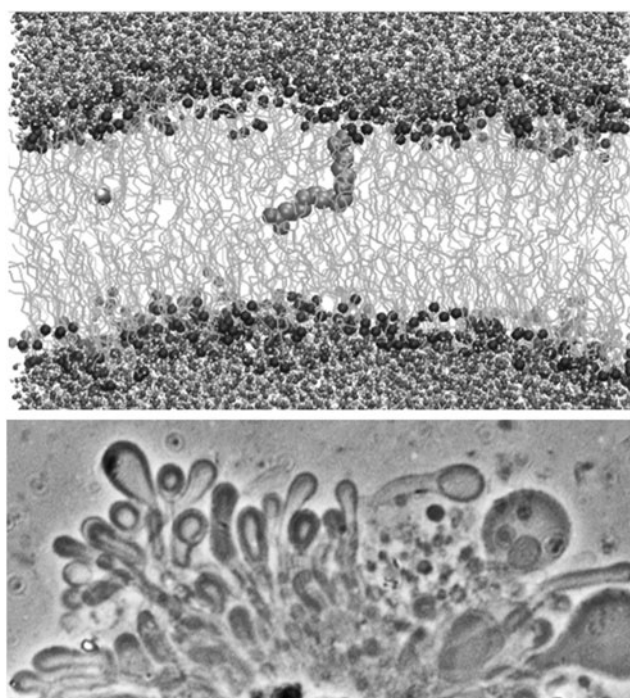


FIG. 3. An MD simulation of a fatty acid membrane is illustrated in the upper image, with one fatty acid shown as a space-filling structure. Light gray indicates hydrocarbon chains, dark gray circles are carboxylate head groups, and water molecules fill either side of the membrane. The lower image shows membranous vesicles emerging from a droplet of dodecanoic acid above its T_m in a Yellowstone hot spring water sample. MD, molecular dynamics. [Milshteyn *et al.* (2018). MD simulation image courtesy of Andrew Pohorille. See Wei and Pohorille (2013) for a detailed description.]

In the early analysis of the components of the Murchison carbonaceous meteorite, Lawless *et al.* (1979) reported that monocarboxylic acids up to 8 carbons in length were present in the mixture, and later studies of Antarctic meteorites extended the chain length to 12 carbons (Naraoka *et al.*, 1999). Monocarboxylic acids compose approximately half the mass of the water-soluble compounds in the Murchison meteorite, about one part per thousand of the total meteoritic mass, which is relatively abundant compared with amino acids (50 ppm) and purines (1 ppm). It was later demonstrated (Deamer, 1985) that some of the amphiphilic compounds were soluble in organic solvents such as chloroform and assembled into membranous vesicles when dried films were exposed to dilute aqueous solutions (Fig. 4). Because the membranous vesicles are fluorescent, they are presumably mixtures of monocarboxylic acids together with some of the polycyclic aromatic compounds known to be present such as pyrene and fluoranthene.

During the course of early studies of Murchison extracts, we were puzzled by the observation that vesicle formation does not occur in seawater. The reason is that the divalent cations in seawater bind to carboxylic acid groups in the extract and cause them to precipitate as aggregated curds rather than forming membranes. The high sodium chloride concentration in seawater also produces an osmotic gradient that inhibits swelling of the amphiphilic compounds into membrane-bounded compartments.

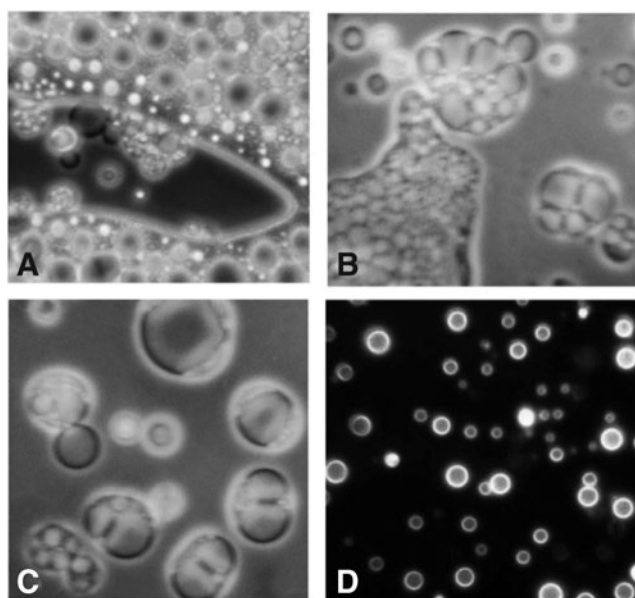


FIG. 4. Assembly of membranous vesicles from amphiphilic compounds extracted from the Murchison meteorite. (A) Initial penetration of water into a dry film. (B, C) Within minutes, the compounds begin to assemble into membranous vesicles having diameters ranging from 10 to 30 μm . (D) The vesicles are highly fluorescent due to their content of polycyclic aromatic hydrocarbons.

Joshi *et al.* (2017) carried out an extensive study on the properties of amphiphilic compounds assembling into membrane structures in water samples from alkaline hot springs in India. Among the different combinations of fatty acids and their derivatives that were evaluated, it was observed that mixtures of fatty acids with their corresponding monoglycerides readily assembled into membranes. The membranes were stable during prolonged heating at a high temperature (75°C), emulative of what such systems might experience in actual hot spring conditions. Some of the mixed membrane systems were unstable when subjected to multiple cycles of dehydration and rehydration in the three different hot spring samples. This was in stark contrast to their behavior in buffered solutions under laboratory simulations, suggesting that certain ionic components of the hot spring water affected stability.

We performed a similar investigation that compared the assembly of dodecanoic acid in hydrothermal water obtained from a hot spring in Yellowstone National Park (Milshteyn *et al.*, 2018) to the same process in seawater (Fig. 5). Membranes were able to emerge from a fluid droplet of the fatty acid (Fig. 5A) because the submillimolar concentrations of mono- and divalent cations in the water did not inhibit the assembly process. In contrast, the more concentrated divalent cations in seawater bind to the carboxylate groups of the fatty acid to form a solid calcium/magnesium soap, seen as aggregated crystals in Fig. 5B.

3.2. Polymerization reactions

After self-assembly of membranes, the second important process related to the origin of cellular life is a mechanism by which polymerization reactions lead to peptides and oligonucleotides. Amino acids are readily synthesized by nonbiological processes, as demonstrated by their presence in carbonaceous meteorites, but a plausible synthesis of nucleotides has not yet been established. For the purpose of this review, we assume that both monomers were available in the prebiotic environment.

Synthesis of proteins and nucleic acids in living cells involves condensation, defined as reactions in which the elements of water are removed from between monomers so that linear strands of polymers are produced. The links are the peptide bonds of proteins and the phosphodiester bonds of nucleic acids. Condensation reactions are not spontaneous, but instead depend on chemical energy. What energy source might have driven bond synthesis in the prebiotic environment?

To answer this question, we can first consider three sources of energy that are used by life today: light energy captured by photosynthesis, the energy made available by electron transport from reduced donors to oxidized acceptors, and chemical energy that drives ordinary reactions of metabolism catalyzed by enzymes. These require highly evolved systems of enzyme-catalyzed metabolism to be used as energy sources, so they would not be available to drive polymer synthesis in the prebiotic era. There is one additional source of energy that would be abundant, however, and this is the chemical potential that becomes available simply by evaporating dilute solutions of potential monomers (Ross and Deamer, 2016). As a result, reactants become extremely concentrated in films of organic material on mineral surfaces, and within those films linking

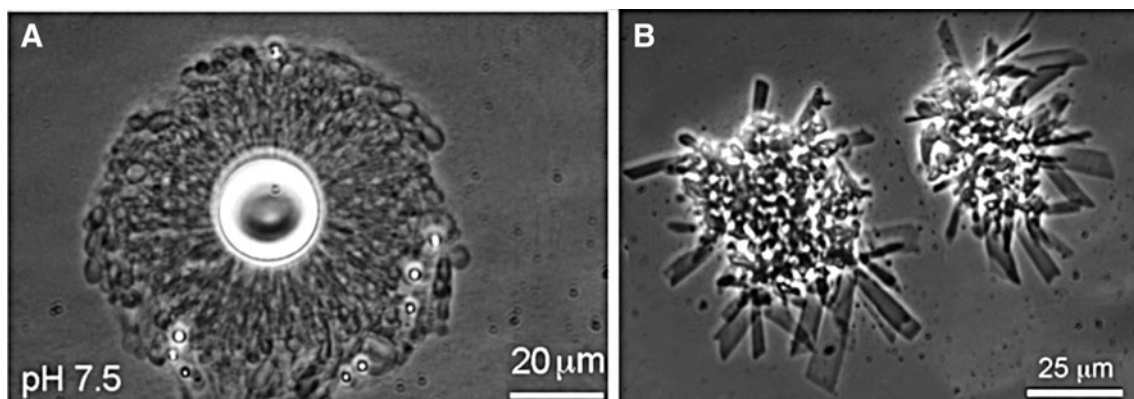


FIG. 5. Comparison of amphiphilic assembly in Yellowstone hydrothermal water (A) and seawater (B). The divalent cation content of seawater causes the fatty acid to form insoluble crystals rather than membranes. From Milshteyn *et al.* (2018).

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bonds can form between the amine and carboxyl groups of amino acids or ester bonds between the phosphate and hydroxyl groups of nucleotides. Elevated temperatures provide the activation energy that allows water to become a leaving group during bond synthesis.

Because dehydration is the simplest source of chemical energy, such reactions have been studied since the 1950s (Fox *et al.*, 1959; Lohrmann and Orgel, 1971). More recent examples of peptide bond formation have been reported by Yu *et al.* (2017), Rodriguez- Garcia *et al.* (2015), and Mayer *et al.* (2018). Polymerization of mononucleotides has also been demonstrated in laboratory simulations (Rajamani *et al.*, 2008; Da Silva *et al.*, 2014; DeGuzman *et al.*, 2014) and shown to be thermodynamically feasible (Ross and Deamer, 2016). However, how confident can we be that reactions occurring in the laboratory can actually work in the prebiotic environment?

One way to address this important question is to test the reactions in contemporary prebiotic analog conditions. This is a virtually unexplored field, probably because researchers are reluctant to abandon the controlled conditions of a laboratory and venture out into the actual conditions they are simulating. One of the first tests was performed on Mount Mutnovsky, Kamchatka, in which a “prebiotic soup” of four amino acids, four nucleobases, phosphate, glycerol, and a membrane-forming amphiphile (myristic acid with 14 carbons), was added to a pool containing ~10 L of boiling water (Deamer *et al.*, 2006). The surprising result was that with one exception, all of the compounds were quickly adsorbed to the clay particles suspended in the water. The exception was the fatty acid, which formed a froth around the edge of the pool in which membranous compartments presumably encapsulated a fraction of the soluble compounds so that they were protected from adsorption to clay.

This experiment inspired a more extensive study in a hot spring site located in New Zealand’s North Island, which illustrates how a prebiotic analog site can be used to test whether results of laboratory simulations can be repeated in natural conditions. Here we present some of the preliminary results in which mononucleotides were exposed to wet/dry cycles at elevated temperatures. Although mononucleotides have been shown to polymerize into oligonucleotides in laboratory simulations, the question is whether such polymerization can also occur in the pH, temperature, relative humidity, and ionic composition of the water in terrestrial hot springs.

One of us (B.D.) visited the Taupo Volcanic Zone (TVZ) near the town of Rotorua in June 2018. A suitable site was found at the Hell’s Gate hydrothermal site to perform experiments. The ionic solutes of an acidic pool (Hells Gate, pH 1.65) and an alkaline pool (Whang, pH 8.1) are presented in Table 8. In general, the concentrations of individual ionic species resemble those of Kamchatka and Yellowstone described earlier, with divalent cation concentrations less than millimolar. The main monovalent cation in the acidic pool is hydrogen ion (22 mM) and this is replaced by sodium (18 mM) in the alkaline pool. The trace element of phosphorus (as phosphate) in the alkaline pool is 2 μ M, which is similar to seawater (3.2 μ M), but phosphate in the acidic pool is 40 μ M, presumably because the more acidic pH dissolves apatite (calcium phosphate) present in the minerals in contact with the water. In contrast, iron and manganese in the alkaline pool (84 and 7.6 μ M, respectively)

TABLE 8. IONIC SOLUTES OF NEW ZEALAND HYDROTHERMAL WATER

Hells Gate			Whang		
Cations (mM)			Cations (mM)		
H ⁺	22 (pH 1.65)		H ⁺ (μ M, pH 8.1)		
Na ⁺	2.4		Na ⁺	18	
K ⁺	0.5		K ⁺	0.56	
Ca ²⁺	0.45 = 0.9 eq		Ca ²⁺	0.014	
Mg ²⁺	0.12 = 0.24 eq		Mg ²⁺	0.001	
Total	26.8		Total	18.6	
Anions (mM)			Anions (mM)		
Cl ⁻	0.4		Cl ⁻	16.75	
SO ₄ ²⁻	13.3 = 26.6 eq		SO ₄ ²⁻	0.46	
Total	27		Total	17.2	
Si	3.7		Si	3.9 ^a	
Trace elements (μ M)					
Hells Gate	Li ⁺ 501	Al ³⁺ 13.7	Fe 0.38	Mn0.05	P40 ^b
Whang	Li ⁺ 0.34	Al ³⁺ 1672	Fe84	Mn7.6	P2

^aThe silicon is expressed as the element in the table, but is present in solution as dissolved silicate.

^bThe valence state of the Fe and Mn is uncertain. The element phosphorus (P) is present as phosphate. Given its pKa of 2.16 and 7.2, the anionic charge will depend on the pH. For instance, in the acidic pool, half the phosphate will be present as H₃PO₄ and half as H₂PO₄⁻, while in the alkaline pool it will mostly be present as HPO₄²⁻.

are far above the seawater concentrations (0.32 and 0.18 μ M). There is also a surprisingly high concentration of trivalent aluminum in the alkaline pool (1.7 mM).

To perform the experiment, we used a 1:1 mole ratio mixture of adenosine monophosphate (AMP) and uridine monophosphate (UMP). These were chosen as a model system of the kinds of RNA monomers that were presumably available in the prebiotic environment, even though a plausible source has not yet been demonstrated experimentally. We also decided to use two monomers that had the potential to form Watson–Crick base pairs, taking into account that prebiotic monomers were unlikely to be available as a pure nucleotide such as AMP alone.

An aluminum block with wells for 48 vials (Fig. 6A) was placed directly into a hydrothermal pool and surrounded by towels to prevent splashes from the boiling pool from encroaching on the block. The block temperature quickly reached 90–93°C. Besides the nucleotide monomers, the mixtures included four different compounds that had previously been shown to promote polymerization: ammonium chloride (NH₄Cl), phosphatidylcholine, lysophosphatidylcholine (LPC), and a 1:1 mixture of lauric acid with its monoglyceride. The mixtures were dried in the laboratory for transportation to New Zealand. At the test site, the first set of 48 vials was hydrated by 0.1 mL of acidic water (pH 1.65) drawn from a nearby hot spring pool and filtered through a sterilizing 0.22 μ m Millipore filter to remove mineral particles and microorganisms. Fumarole vents near the pool also exposed the mixtures to the high relative humidity and sulfur dioxide present in the vent gases that drifted over the vials.

Despite the high relative humidity, the solutions in the vials partially dried in 7 min and were fully dehydrated

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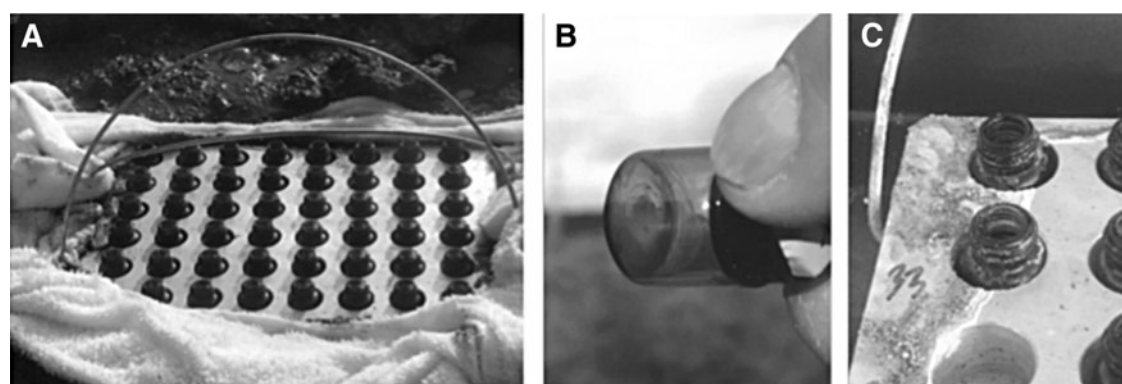


FIG. 6. (A) Vials were housed in an aluminum block set into a hydrothermal pool. (B) A lipid film can be seen in a vial during the dry phase of a wet/dry cycle. (C) The block and vials are shown after 4-h and four wet/dry cycles.

within 15 min. The dried films were visible through the glass (Fig. 6B). After dehydration, the samples were left in place for 35–40 min to allow polymerization to occur within the concentrated films, followed by rehydration by adding 0.1 mL of the acidic hot spring water to each vial. After four cycles, the block was removed and the vials were capped for transport. Figure 6C shows the condition of the vials and block after four hydration/dehydration cycles. Although a mineral deposit formed where the hot spring water evaporated on the block, none of the vials was contaminated despite their close proximity to an active hydrothermal environment.

After the vials were returned to the laboratory, RNA-like products were isolated by a standard method, in which 70% ethanol precipitated the polymers. Figure 7 shows yields of RNA-like polymers synthesized in the acidic hot spring water.

The pyrimidine nucleotide UMP had the highest yields, followed by the mixture of AMP and UMP, while three of the four AMP samples had the lowest yields. The total mass of nucleotides initially present in four vials was 1400 μg , so the highest percent yield was 23%, and the lowest was 4%.

We performed gel electrophoresis of the products and compared the gel patterns with those of laboratory simulations. Figure 8A shows a typical laboratory gel with a ladder of known lengths, compared with a synthetic polynucleotide composed of polyadenylic acid (polyA) and polyuridylic acid (polyU) mixed in a 1:1 ratio of the bases. The lane on the right shows the band of a polymeric product produced by cycling a mixture of AMP and UMP in the presence of LPC. The products are stained with ethidium bromide, a fluorescent dye that binds to nucleic acids by intercalation between stacked bases. (The images have been inverted so that fluorescence is

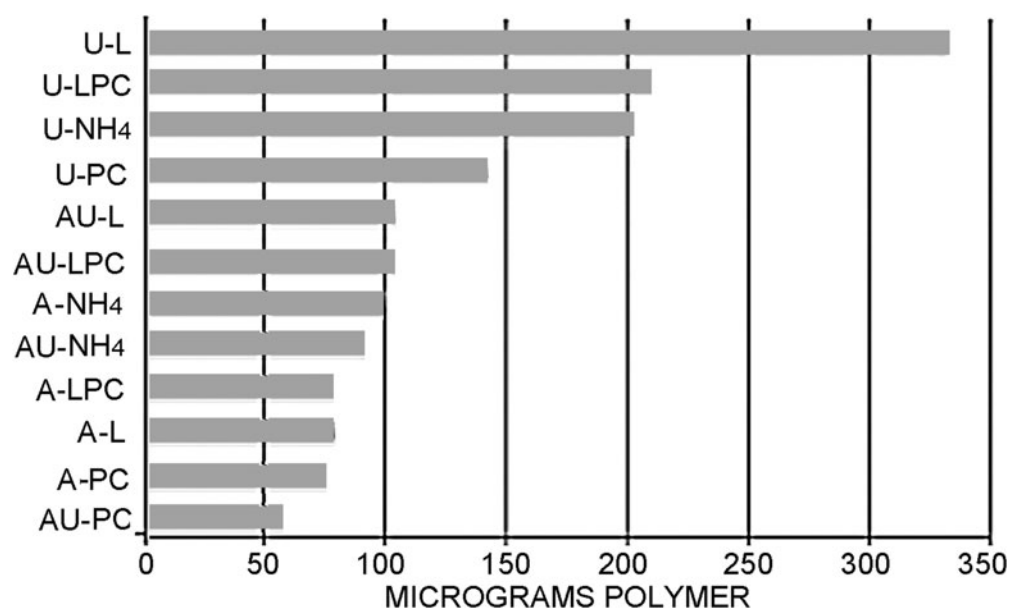


FIG. 7. RNA-like polymers were precipitated in ethanol. Each bar shows total combined yields in micrograms from four vials exposed to four wet/dry cycles using acidic hot spring water. A, U, and AU represent 10 mM AMP, UMP, and 1:1 AMP + UMP present in a given vial as monomers. Promoters known to promote polymerization were also in the mixture: PC (10 mM), LPC (10 mM), NH₄ (100 mM). These are initial concentrations that increase during evaporation. L is a 1:1 mixture of lauric acid and lauroyl monoglyceride (initial concentration 10 mM), a model system for prebiotic amphiphilic compounds able to assemble into membranes. AMP, adenosine monophosphate; LPC, lysophosphatidylcholine; NH₄, ammonium chloride; PC, phosphatidylcholine; UMP, uridine monophosphate.

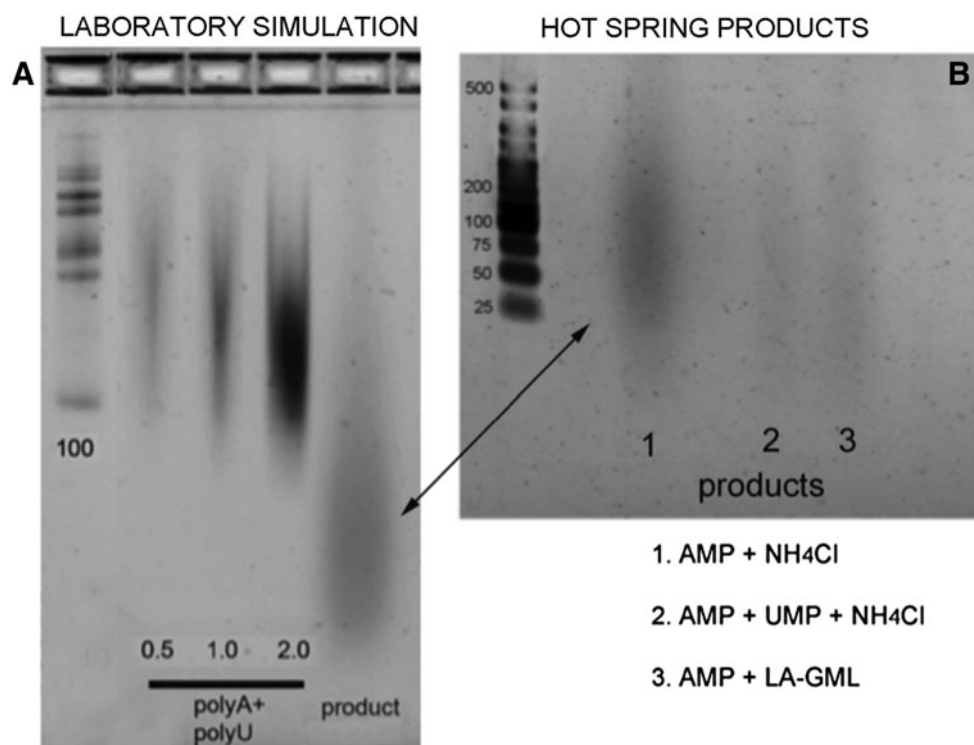


FIG. 8. Comparison of polymer products from four wet/dry cycles in a laboratory simulation and four wet/dry cycles using acidic hydrothermal water in the Hell's Gate hydrothermal system near Rotorua, New Zealand. The polyA+ polyU lanes in the left hand image represent a positive control to demonstrate ethidium bromide staining of a known helical structure formed by a 1:1 mixture of polyA and polyU. The images are inverted for clarity so that fluorescence appears dark against a light background. polyA, polyadenylic acid; polyU, polyuridylic acid.

shown as dark against a light background.) The ladder in the gel on the left starts with a stained band 100 nucleotides (nt) long, so the polyA-polyU RNA ranges from 100 to 200 nt in length. The product polymer is revealed as a streak from ~20 to >100 nt on the same scale. Similar streaks in that range can be seen in the products synthesized by cycling mononucleotides in acidic water of a New Zealand hot spring (arrow).

We used phase and fluorescence microscopy to observe the structures that assemble when a dry mixture of amphi-

philic compounds is exposed to hot spring water at a temperature above T_m of 46°C. Rather than the highly evolved phospholipids of contemporary life, we chose to use a 1:1 mixture of lauric acid and its monoglyceride, which is a more plausible model system of prebiotic membrane-forming amphiphiles. Figure 9A shows worm-like structures called myelin figures. These are composed of multilamellar cylindrical structures that emerge when dry amphiphiles are exposed to water. We also tested whether encapsulation was possible

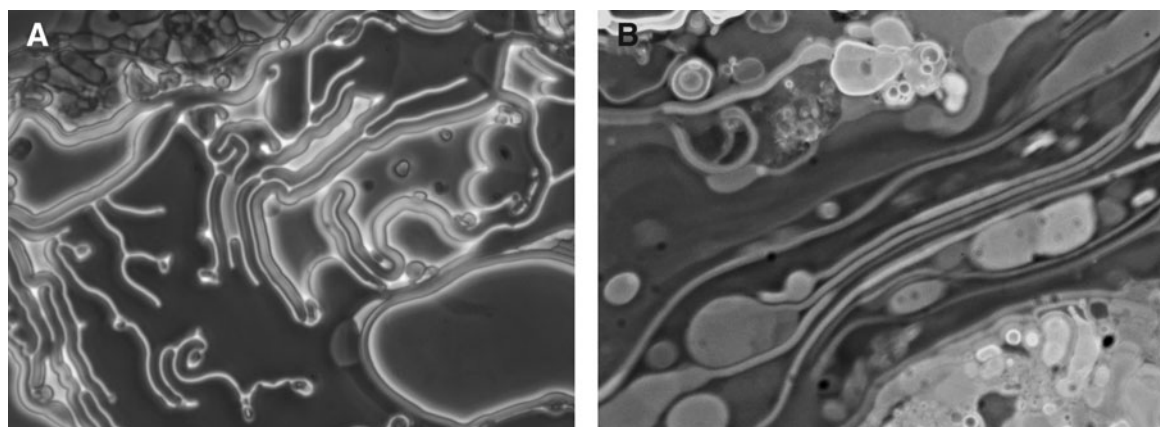


FIG. 9. (A) A phase micrograph of a 1:1 mixture of lauric acid and its monoglyceride dried on a microscope slide, then hydrated with 20 µL of acidic hot spring water and heated above 46°C. The amphiphiles assembled into tubular myelin figures composed of multilamellar concentric layers. (B) A fluorescence micrograph of the same lipid mixture dried in the presence of a nucleic acid ladder. Instead of myelin figures, large fluid vesicles formed that contained nucleic acid stained with acridine orange dye.

in hydrothermal spring water. Figure 9B shows the same amphiphilic mixture but dried in an approximate 1:1 ratio by weight of a DNA ladder with lengths from 50 to 500 nt. We preferred a ladder rather than biological DNA because a mixture of short strands is a preferred model of nucleic acids produced by nonenzymatic polymerization reactions. The inclusion of DNA had a remarkable effect on the amphiphilic structures. Instead of myelin figures, the membranes were fluid compartments containing DNA stained by the addition of acridine orange dye. This is the first evidence that a nucleic acid polymer can affect the structure and properties of amphiphilic compounds as they assemble into compartments that contain the polymer.

We conclude that polymerization reactions observed in laboratory simulations also occur in water from an acidic hot spring undergoing wet/dry cycles. The reaction is not inhibited by the high relative humidity or the sulfur dioxide in the atmosphere near the hot spring pool. If polymers are synthesized in the presence of amphiphilic compounds, wet/dry cycles also serve to encapsulate the polymers and form protocells.

3.3. Future direction of hot spring field work

Although the results described above are preliminary, they are clearly encouraging. We present them to demonstrate that it is possible to test the common assumption that laboratory results reflect chemical reactions expected to occur in the prebiotic environment. They also show that testing a hypothesis is not restricted to laboratory conditions but can be carried out in prebiotic analog sites.

One limitation of the approach is that the experiments were necessarily carried out in glass vials to contain small samples of reaction mixtures and then transport them back to the laboratory for analysis. The next question is whether polymerization can also occur when the wet/dry cycles are carried out in hydrothermal water exposed to cycling on mineral surfaces. To this end, in 2020, we plan to return to the hydrothermal site in the TVZ and place larger amounts of the reagent mixtures in mineral depressions or side pools that are directly in contact with the hydrothermal waters. Such environments can be

found in a wide range of hot spring-infused sediments in creek beds (Fig. 10A) and pools found on the outflow channels of sinter aprons (Fig. 10B) [see Schinteie *et al.* (2007) for a detailed description]. Samples will be taken after multiple wet/dry cycles and then returned to the laboratory for microscopic examination and analysis of potential polymer products.

4. Conclusions

We can first ask whether the hypothesis proposed in the introduction has value in guiding research. We believe that it does have value, because it forced us to step out of the laboratory to determine whether laboratory results in carefully controlled conditions are sufficiently robust to work in a prebiotic analog environment. Furthermore, by taking into account the requirements for self-assembly and condensation reactions, we could compare the physical and chemical properties of hydrothermal water in alkaline vents and hot springs and weigh evidence related to plausible conditions for the origin of life. Hydrothermal water in both alkaline vents and terrestrial hot springs is in a temperature range that is tolerated by life, as demonstrated by the thermophilic microorganisms that thrive in both environments. Life has also managed to adapt to the high and low pH ranges of vents and fields, and to fresh water and extreme saline concentrations. However, there are significant differences in the two sites that affect the processes required for life to begin, so it does not follow that such conditions will be equally conducive for the origin of life on the prebiotic Earth. Those differences are summarized below. Our intention is not to be critical of the processes proposed to occur in hydrothermal vents, but instead to encourage experimental studies that will test whether polymerization and encapsulation can occur in vent conditions, as we have described here for hot spring conditions,

4.1. Ionic concentrations and self-assembly of amphiphiles

The concentration of ions in water associated with hydrothermal vents is basically that of the seawater, including



FIG. 10. (A) Hydrothermally influenced sediments at Parariki Stream, New Zealand. (B) Sinter aprons (arrows) surrounding outflow pools near Te Kapua at Orakei Korako, New Zealand.

relatively high concentrations of divalent cations. Numerous laboratory studies have demonstrated that divalent cations bind to carboxylate groups of amphiphilic compounds such as fatty acids and inhibit assembly of membranous compartments. In contrast, divalent cation concentrations are relatively low in hydrothermal spring water, and membrane assembly has been demonstrated both in the laboratory and in the field.

4.2. Sources of organic compounds

Proposals that life began in vent conditions suggest that organic compounds would be synthesized by reduction of carbon dioxide, with hydrogen as a source of reducing power. This reaction is hypothetical and has not yet been convincingly demonstrated either by sampling vent water or in the laboratory. Furthermore, because organic compounds would be diluted if they fell into the early ocean, it seems unlikely that they could accumulate and become sufficiently concentrated in hydrothermal vents to undergo synthetic reactions related to the emergence of life. In contrast, organic compounds falling on volcanic land masses would be flushed into small volumes of hot spring water and further concentrated by evaporation.

4.3. Cycles of hydration and dehydration

Wet/dry cycles in the fresh water of hot springs concentrate potential reactants and provide chemical potential to drive polymerization reactions. Such cycles cannot occur in hydrothermal vent conditions.

4.4. Thermodynamic feasibility of condensation reactions

Research cited earlier in this essay demonstrated that RNA-like polymers are synthesized in simulated hot spring conditions. The polymers resemble RNA in chemical composition and biophysical properties but are referred to as “RNA-like” because both 2′-5′ and 3′-5′ linking bonds are present, while biological RNA incorporates only 3′-5′ bonds. Here we report preliminary results that RNA-like oligomers can also be synthesized in hot spring conditions. We conclude that polymerization readily occurs in dilute solutions that undergo wet/dry cycling. The thermodynamic feasibility of this reaction has been analyzed by Ross and Deamer (2016), but there is a substantial thermodynamic barrier to condensation reactions in bulk seawater because condensation reactions can only be thermodynamically feasible in aqueous solutions if the reactants are chemically activated. As yet, there is no experimental evidence for a plausible activating mechanism in seawater.

4.5. Light energy

Sunlight cannot penetrate to the depths at which hydrothermal vents occur. In contrast, abundant light energy is available in terrestrial hot springs, enabling photochemical reactions required for a primitive version of photosynthesis.

4.6. Encapsulation

For cellular life to begin, there must be a process by which functional polymers become encapsulated in membranous compartments. Such a process has long been known in lab-

oratory simulations and more recently reported in water samples obtained from Yellowstone hot springs (Milshteyn *et al.*, 2018). Encapsulation of polymers has not been demonstrated in the seawater of hydrothermal vent conditions.

From these considerations, in our view, the weight of evidence supports terrestrial hot springs as the more conducive site for the emergence of protocells. This conclusion has implications for the search for life on other potentially habitable planetary bodies in the Solar System. For instance, we have argued that if the origin of life requires volcanic land masses and fresh water undergoing wet/dry cycles, then it is unlikely that life could begin on icy moons such as Enceladus and Europa (Deamer and Damer, 2017). On the contrary, the Spirit rover and more recently Curiosity have provided clear evidence for volcanism and shallow seas on Mars, so it is reasonable to propose that hydrothermal sites such as Columbia Hills should be targets for future life-detection missions to Mars.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

AMP = adenosine monophosphate

LPC = lysophosphatidylcholine

polyA = polyadenylic acid

polyU = polyuridylic acid

TVZ = Taupo Volcanic Zone

UMP = uridine monophosphate

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