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Perspective

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Physical non-equilibria for prebiotic nucleic acid chemistry

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Abstract

The prebiotic replication of DNA and RNA is a complex interplay between chemistry and the environment. Factors that have direct and indirect effects on prebiotic chemistry include temperature, concentration of monovalent and bivalent ions, the pH of water, ultraviolet irradiation and the presence of gaseous CO_2. We discuss various primordial conditions to host the first replication reactions on the early Earth, including heated rock pores, hydrothermal vents, evaporating water ponds, freezing–thawing ice compartments, ultraviolet irradiation and high CO_2 concentrations. We review how the interplay of replication chemistry with the strand separation and length selectivity of non-equilibrium physics can be provided by plausible geo-environments. Fast molecular evolution has been observed over a few hours in such settings when a polymerase protein is used as replicator. Such experimental findings make us optimistic that it will soon also be possible to probe evolution dynamics with much slower prebiotic replication chemistries using RNA. Our expectation is that the unique autonomous evolution dynamics provided by microfluidic non-equilibria make the origin of life understandable and experimentally testable in the near future.

Sections

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Introduction

To understand possible mechanisms for the origin of life, it is essential to study the environmental conditions that could generate and sustain the first stages of molecular evolution. These boundary conditions were defined by the geological conditions and processes of the very early Earth, probably in the Hadean eon after the appearance of liquid water about 4.4–4.0 billion years ago[1–3]. Various geological settings with and without the presence of limited surficial landmass are considered plausible environments at the late Hadean and early Archaean. Some of the most studied settings are shown in Fig. 1. Each of these can host one or more non-equilibrium mechanisms that make it particularly appealing in the context of Hadean molecular evolution.

Such geological systems are sources of local non-equilibrium conditions, driven by the slowly cooling early Earth under a young faint Sun. These non-equilibria are essential because, according to entropy arguments, life could not have originated under equilibrium conditions[4,5]. The origin of life needs a high level of self-organization, high concentration of reagents and a continuous influx of energy to be able to evolve towards more complex systems. Without a means of lowering the entropy, this process becomes impossible, and the system slowly reaches equilibrium and decays into a dead soup. For example, under equilibrium, RNA and DNA oligomers dilute by diffusion, reduce their length by hydrolysis and cannot replicate sequence information by polymerization through activated molecules as these cannot be replenished. The necessary non-equilibria — such as concentration gradients, salt and pH cycles, irradiation or temperature differences — actively drive the system towards a continuous and dynamic self-organization[6–8] and could be provided by external forces such as geothermal heating, solar and isotopic irradiation, day–night cycles and atmospheric phenomena. The search for settings that are able to deliver continuous forces to maintain the chemical system out of equilibrium is therefore important.

Investigating the early Earth environment that may have provided the initial conditions for the emergence of life also offers inspiration for research into the question of extraterrestrial life. Although some astrophysical space missions, such as NASA's Transiting Exoplanet Survey Satellite (TESS[9]), focus on searching for Earth's analogues, there is increasing evidence that the sort of out-of-equilibrium environments reviewed in this Perspective may also be common on celestial bodies that are far from being Earth analogues. For instance, even if most rocky planets in the habitable zone around cooler (M) stars that were targeted by the Kepler mission[10] are expected to be tidally locked (that is, always showing the same side to their parent star), they are still likely to have something resembling day–night cycles driven by weather systems, which could in turn drive wet–dry cycles in some regions on their surface. Extreme examples of possible habitable worlds that have conditions far from those of Earth are moons around giant planets, like those in our Solar System (Io, Europa, Titan), in extrasolar systems or, even more exotically, moons around free-floating planets[11]. The huge investment in space missions aiming at atmospheric studies of extraterrestrial and extrasolar bodies, due to be launched within the next two decades, will strongly benefit from origins of life research that studies this complex process on Earth.

In this Perspective, we survey commonly studied geological settings in the Hadean Earth, before discussing how the non-equilibrium conditions hosted by these settings could drive molecular evolution.

Hadean Earth geological conditions

Wet–dry cycles in evaporating water ponds

In the past, the most often cited setting for the origin of life has been Darwin's "warm little pond" of fresh water on land[12,13], where reagents in the organic soup can become concentrated enough for chemical reactions to occur (Fig. 1a). Day–night, seasonal temperature, or weather oscillations can be imagined to lead to fluctuating water levels and

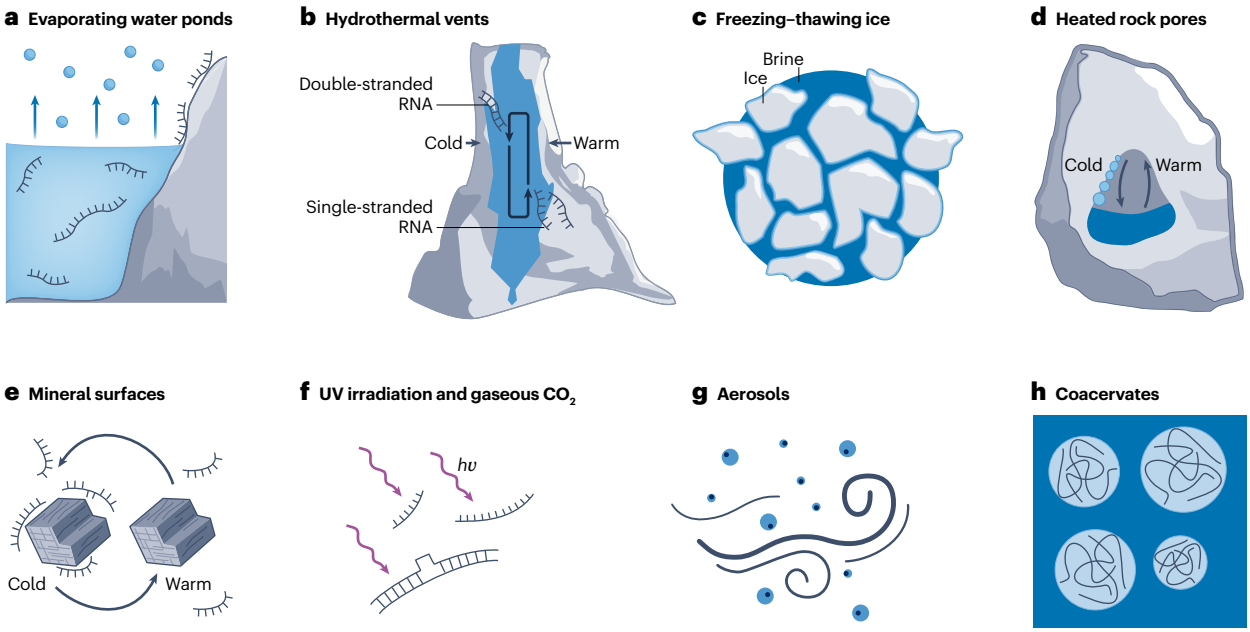

Fig. 1 | Proposed primordial geological settings for fluctuations in temperature, salts and pH, ultraviolet irradiation and high CO_2 concentrations. **a**, Evaporating water ponds on surficial land. **b**, Submarine hydrothermal vents. **c**, Freezing–thawing cycles in ice. **d**, Heated rock pores. **e**, Mineral surfaces. **f**, Atmospheric ultraviolet radiation and gases. **g**, Aerosols. **h**, Liquid–liquid phase separation, forming coacervates. These systems are chemically rich and offer a variety of non-equilibrium forces that could have enhanced the prebiotic chemical reactions and driven the molecular evolution.

wet–dry cycles. This system continuously generates fluctuations in the concentrations of molecules, reagents and salts, which can become exceedingly high in the completely dry state. In addition, the pH, which depends on the chemical properties of the concentration-varying species, changes during evaporation. The chemical complexity of wet–dry cycles can enhance those chemical reactions that benefit from high concentrations, including the synthesis of canonical and non-canonical nucleosides[14–16], the phosphorylation of prebiotic molecules[17], the formation of peptide bonds between amino acids[18], the ester bond formation of sugar monomers into polymers[19], the polymerization of RNA from single nucleotides[20,21], and the emergence of ribozymes and transfer-RNA-like structures[22], among others. However, because nucleobases rapidly photodissociate during illuminated dry periods and are lost into pond seepage during the wet periods, it is possible that the synthesis of nucleotides and the subsequent RNA polymerization might require a few wet–dry cycles[23].

Chemical and temperature gradients in hydrothermal vents

Later, submarine hydrothermal vents (Fig. 1b) could have played a role as habitats for the first forms of life[24–27]. These systems are chemically rich and contain dissolved chemicals from interactions with the newly formed oceanic crust and the underlying hydrothermal system. They are expected to host pH gradients and drive a variety of redox reactions[28]. Moreover, rock cavities in their proximity are subject to steep temperature gradients, which induce convection and thermophoresis, cycling the solutes between hot and cold temperatures and inducing their accumulation[29,30]. For DNA, thermal replication and thermal accumulation could be combined[31,32]. A considerable number of chemical reactions of smaller metabolic molecules have been demonstrated to occur in these systems: redox reactions involving iron, sulfate and methane[28]; the interconversion and formation of amino acids[33,34]; RNA oligomerization[35]; and DNA accumulation and polymerization[31,36,37]. However, the absence of wet–dry cycles within hydrothermal vents has limited RNA polymerization in these systems to short oligomers[23,35]. Moreover, the high temperatures of the hydrothermal vents pose a problem for hydrolysis reactions, which may degrade the newly formed polymers[38], but could also open new pathways for protometabolic reactions[39].

Freezing–thawing ice in brine microchannels

Another geological setting to consider is ice (Fig. 1c), which should not be excluded at the poles or at exposed landmass on the early Earth, or in the scenarios of variable CO_2 partial pressure and the faint Sun hypothesis[40,41]. During the formation of ice from seawater (or other solute-rich solutions) water freezes to create ice crystals of pure water, while the remaining solute-rich liquid brine progressively concentrates in interstitial microchannels with a size of several micrometres, subjecting the molecules to oscillatory conditions under freeze–thaw cycles[42]. At lower temperature, when the salt solubility limits are reached, salts can precipitate and create crystals. Degassing leads to gas bubbles, which are incorporated into the brine channels and the ice. Gradients of pH between the brine and the ice are created[43]. The ice's eutectic phase and freezing–thawing seawater offer a large chemical variety that has been shown to accelerate many chemical reactions involving RNA. Some notable examples are the templated polymerization of aminonucleotides[42,44]; the assembly of active ribozymes[45]; the ribozymatic replication of RNA[46]; and a one-pot pathway from nucleotide activation to non-enzymatic RNA extension after repeated freeze–thaw cycles[47].

Microscale water cycles in rock pores

Heated rock pores containing gas and water (Fig. 1d) are another geological setting that was probably abundant when life emerged, and the prebiotic relevance of which has been explored[48,49]. This system exists in the proximity of heat sources (such as magmatic activity in general, active volcanic regions or hydrothermal systems), with gas bubbles arising from magma degassing or from the incorporation of air. The differentially heated surfaces inside the pore trigger a water cycle at the microscale, generating temporary salt and pH fluctuations, as well as wet–dry cycles. This setting could host the crystallization of ribose, RNA phosphorylation and gelation, ribozyme catalysis and the formation of lipid vesicles[49]. The pH and salt fluctuations of the water cycle also promote strand separation at low temperatures[48,50]. Moreover, it has been shown that CO_2-acidified water drives DNA replication (albeit in an enzymatic manner), providing evidence for sequence evolution towards longer strands with an AT:GC composition that depends on the physico-chemical conditions of the environment[51,52].

Charged surfaces and leaching on mineral surfaces

The interaction of mineral surfaces with water on the early Earth (Fig. 1e) is almost inevitable, and part of all the systems discussed above. A variety of minerals form the walls and surfaces of hydrothermal vents, build the rocks hosting pores, and form the sediments within water ponds. The minerals offer a variety of effects on prebiotic molecules and chemistries and probably played a major role in molecular evolution[53]. Different types of minerals, rocks and glasses have been reported to enhance various chemical reactions: borate minerals for the synthesis of RNA components such as ribose[54]; phosphate minerals for the phosphorylation of nucleosides[55]; the synthesis of DNA and RNA oligomers on montmorillonite and hydroxyapatite[56–58]; the regulation of ribozymatic catalytic activity by tholeiitic basalt surfaces[59]; RNA polymerization promoted by montmorillonite and amino acids[60]; the synthesis of polyribonucleic acid promoted by rock glasses[61]; and the preferential accumulation of long RNA molecules[62], among other examples. Mineral–water interactions also result in ions and molecules leaching out of the minerals over time by dissolution, ion exchange and surface complexation reactions[63]; thus minerals can act as buffers for aqueous solutions[64] or provide ions to ribozymes and oligonucleotides[59], depending on the type of mineral used and the ions it contains. In addition, clay mineral surfaces, in particular those of montmorillonite, have been shown to interact with hairpin ribozymes to protect them from degradation by ultraviolet (UV) irradiation, retaining the catalytic activity of the ribozymes for longer times[65].

Irradiation by high-energy photons

Atmospheric factors such as UV irradiation and the concentration of gaseous CO_2 also have major effects on prebiotic chemistry (Fig. 1f). Without access to molecular oxygen and the resulting ozone layer, the surface of the early Earth was reached by UV radiation with wavelengths as low as 200 nm (ref. [66]). At 260 nm, the solar photon flux on the surface reached intensities of 10^{12}–10^{13} photons per square centimetre per second (ref. [66]). Owing to the high photon energy in the range of 200–300 nm, more complex organic compounds such as nucleobases could be damaged[67]. Although in aqueous solutions dissolved metal ions can shield this radiation (such as bromide for wavelengths $\lambda < 220$ nm, or Fe^{2+} for $220 < \lambda < 300$ nm), in natural waters on the early Earth the penetration depth of 200–360 nm light reached up to several metres[68]. Therefore, it is essential to consider the influence of UV light as a physical selection pressure on prebiotic chemistry.

The canonical nucleobases (A, C, G, T and U) are particularly resistant to UV irradiation[69] because they can convert the absorbed energy into heat on sub-picosecond timescales[70,71]. In contrast, other precursors, such as 2-aminooxazole or 2-aminoimidazole, are more susceptible to UV damage[72,73]. Ultraviolet radiation is not only destructive but can also catalyse the interconversion of nucleosides through photo-induced hydrolysis[74], and drive redox reactions to produce nucleoside, amino acids and lipid precursors[75,76].

Besides UV radiation, other ionizing radiations such as solar X-rays[77], solar protons[78] and gamma rays from radioisotopes[79] (for example, ^{40}K, ^{232}Th, ^{235}U and ^{244}Po) were abundant on the Hadean Earth surface and inside nuclear geysers[80] (underground natural nuclear reactors). Ionizing radiation has been historically shown[81] to drive various chemical reactions, such as the formation of amines, amino acids and a variety of organic compounds (such as alanine, glycine, formic acid, ammonium acetate and urea). More recently, it has been shown how ionizing radiation can be an important energy source in prebiotic synthesis, for the formation of formamide[82], sugars and nucleosides[78,83], and to generate chemical diversity by radiolytically produced prebiotic precursors[84]. Ionizing radiation can also aid the genetic mutations that are necessary for evolution[85]. However, it can also disintegrate biomolecules by radiolysis and trigger the formation of radicals that have detrimental effects on the origin of life[86].

Gaseous CO_2 leading to water acidification and mineralization

The pressure of gaseous CO_2 in the Hadean atmosphere has been estimated to have been in the range of 0.01–10 bar, much higher than today ($\sim 3.5 \times 10^4$ bar)[40,41,87,88]. When CO_2 is absorbed into water, it leads to the formation of carbonic acid, bicarbonate and carbonate, and therefore to water acidification[89]. In the absence of additional buffers (for example in condensation water formed during the water cycle), the pH is directly proportional to the partial pressure of CO_2 (ref. [90]). It has been argued that surface waters on the Hadean Earth were therefore probably acidic, which would have profound implications for the prebiotic chemistries[91,92], for the molecular evolution of oligonucleotides[51] and for the climate of the early Earth[93]. An atmosphere rich in CO_2 could also have had a role in the release of phosphate from phosphate minerals such as apatite; the increased solubility of apatite in CO_2-acidified water could strongly increase the availability of phosphate at concentrations and pH levels relevant for prebiotic syntheses[94].

Compartmentalization and drying of aerosols

Another system of prebiotic relevance is atmospheric aerosols (Fig. 1g), generated by wind upon the breaking of waves at the ocean surface, bubble-bursting or geysers[95]. The aerosol particles, the size of which is determined by aerodynamic drag, surface tension and gravity, have a high surface-to-volume ratio. The organic molecules and long-chain surfactants rearrange at the aerosol–wind interface and form a spherical monolayer that encloses an aqueous core, a configuration known as the 'inverted micelle' model[96,97]. This structural rearrangement implies that most of the organic content of the particle resides at the surface, increasing the concentration of the molecules in that region. During their passage through fluctuating fields of humidity and temperature in the atmosphere, the aerosols rapidly lose water from the central core until a full external organic monolayer is formed[98]. The chemical variety, size and the surfactant wall give aerosols strong similarities to simple cells. Reactions such as polymerization, OH oxidation reactions, compartmentalization and the formation of various organic molecules have been demonstrated to be enhanced in the crowded and dehydrated interior of aerosol particles, which also experience pH changes during their lifetime[96,99–101].

Liquid–liquid phase separation and compartmentalization

Coacervation, the liquid–liquid phase separation of oppositely charged polyelectrolytes in water (Fig. 1h), has been hypothesized to have played a major role during the origin of life. The membrane-free protocells produced by coacervation can spatially localize molecules, aid fatty acid bilayer assembly[102], selectively concentrate a range of different molecules[103–105] and tolerate catalytic reactions involving RNA and ribozymes[106,107]. Coacervate droplets of polypeptides, polysugars and oligonucleotides are highly dynamic and form with heterogeneous chemical identity[108]. Interesting effects arise when they are put in a fluctuating environment, such as microfluidic water cycles inside heated rock cavities. There, the fluxes generated by moving water rapidly make the coacervate droplets fuse together, divide and fragment[109].

Non-equilibria to drive molecular evolution

To test the physical non-equilibrium constraints described above in a chemical context, many example systems are available. These examples are not necessarily limited to canonical ribonucleotides, as non-canonical systems could have been precursors to an RNA world or other hypothetical scenarios[14,110–112]. However, because the properties of canonical ribonucleotides are exceptionally well studied and many deep analytical methods such as high-throughput sequencing have been established, in this Perspective we focus on how combinations of physical non-equilibrium systems can enable RNA-related reactions and push their sequence space by applying external selection pressures. That said, it is still a matter of debate as to which molecule came first: RNA, DNA, proteins, RNA–protein hybrids, or their coexistence; and which function they would carry[113–117].

Strand separation under temperature, salts and pH synergism

One of life's most important features is the ability to continuously copy its genetic blueprint, stored in DNA and RNA. Its replication requires conditions in which the copied product strand can detach from its template, which is typically problematic under standard conditions. This difficulty is also known as the strand separation problem[118]. The issue arises from the fact that long RNA duplexes have a very high melting temperature (sometimes even higher than the boiling point of water), and their accurate copying would generate a dead-end duplex product. Moreover, the rate of strand reannealing at high strand concentrations is orders of magnitude faster than the current copying chemistries. Therefore, it is considered central to understand how the previously discussed geophysical systems can offer kinetic, non-equilibrium solutions to this problem.

The temperature, salts and pH fluctuations that occur in geological settings are subject to various geological processes of the early Earth. As discussed below, they can act synergistically to induce strand separation at moderate physico-chemical conditions. Figure 2a–d summarizes the main determinants of the duplex stability of oligonucleotides. Temperature, concentration of monovalent and bivalent ions[119], and pH[120] determine whether a specific oligonucleotide sequence is in the single-stranded or double-stranded conformation[121]. A single one of these factors is usually not enough to trigger strand separation without reaching extreme values, which could damage RNA and chemical reagents (as is the case with very high temperatures), or could be incompatible with prebiotic chemistries (such as for very low salts or pH). Instead, when all these factors act in synergy, a substantial

strand separation can be achieved in moderate regimes that can be compatible with many replication chemistries (Fig. 2e).

The environment drives sequence evolution

Several reports have shown how the environment can influence the final sequence composition and length of replicated, ligated or polymerized sequences[51,52,122–124]. In a simulated replicative environment of the RNA world, the sequences adapted to the melting conditions of an acidic microscale water cycle[51]. The pH, salts and temperature fluctuations created specific regimes of melting conditions. Only the sequences with intermediate duplex stability could continuously melt and reanneal, and thereby undergo repeated replication cycles. This effect strongly biased the resulting sequence length and ATGC content, creating long sequences whose AT/GC fraction correlated with the melting conditions. For example, mild denaturing conditions promoted the replication of sequences rich in A and T, whereas stronger ones promoted the creation of sequences richer in G and C (Fig. 3a). This effect derives from the fact that the AT/GC ratio of a duplex oligonucleotide sequence is a major factor that determines its stability against denaturation[125].

On an early Earth, therefore, different environments could host pools of sequences enriched in specific nucleotides. This differentiation would strongly reduce the occupied states in the sequence space[126] and would render the spontaneous emergence of particular sequence biases or motifs far more possible: an essential feature for the emergence of ribozymes in an evolving RNA world[127,128]. The sequence space of long oligonucleotides is immense. Without a mechanism to selectively reduce the sequence space, any sequence bias is likely to remain hidden and not emerge. The non-equilibrium features discussed above are able to circumvent this limitation, selecting specific subsets of sequences of defined nucleotide composition[51,129]. Moreover, these observations could give an explanation to the paradox of the dual roles of RNA in the RNA world, as both information carrier and catalyst[130]. These dual roles require the RNA to have good templating ability and stable folding, which are in conflict with each other. However, an open-ended replicating system drives the evolution of sequences with intermediate stability, whose melting temperature (T_m) is close to the environmental temperature. In that temperature regime, sequences can repeatedly fold and unfold, satisfying the dual role of RNA in the RNA world.

Ribozymes under non-equilibria

The catalytic activity of ribozymes is strongly influenced by salts, temperature and pH, because these factors directly affect their folding[131–134]. For example, RNA-stabilizing conditions (that is, high salts and low temperature) can shift the equilibrium of the hairpin ribozyme towards ligation. Conversely, destabilizing and melting conditions shift the equilibrium towards cleavage[123]. It is therefore an attractive hypothesis that fluctuating environmental conditions could tune the activity of ribozymes and introduce biases into a pool of replicating sequences, to drive molecular evolution towards a net direction (Fig. 3b).

In addition, the interactions of ribozymes with mineral surfaces insert an additional layer of regulation to their catalytic activity. As ions are liberated from minerals, heat flows actively alter the ionic salt ratios (for instance, the ratio of Mg^{2+} to Na^{+}) to an extent that enables key ribozyme activities in otherwise challenging solution conditions[59].

Kinetics-mediated sequence evolution

Salts, temperature and pH also affect the kinetics of annealing of complementary strands. The annealing process is slower for lower salts and pH[48,135–140]. Therefore, denaturing conditions increase the time required for strand annealing. With a limited time window available, only the sequences with a faster and more accurate annealing (such as those with fewer mutations) would be able to remain active during the replication process. The sequences that are unable to anneal in that time stall and die out.

This mechanism can trigger sequence-selective processes that bias the sequence pool of a replicative system (Fig. 3c). One example is the selection of sequences containing fewer mutations (kinetic error filtering[124]), a sort of non-enzymatic proofreading method. Another example is a self-enhancing sequence selection that arises

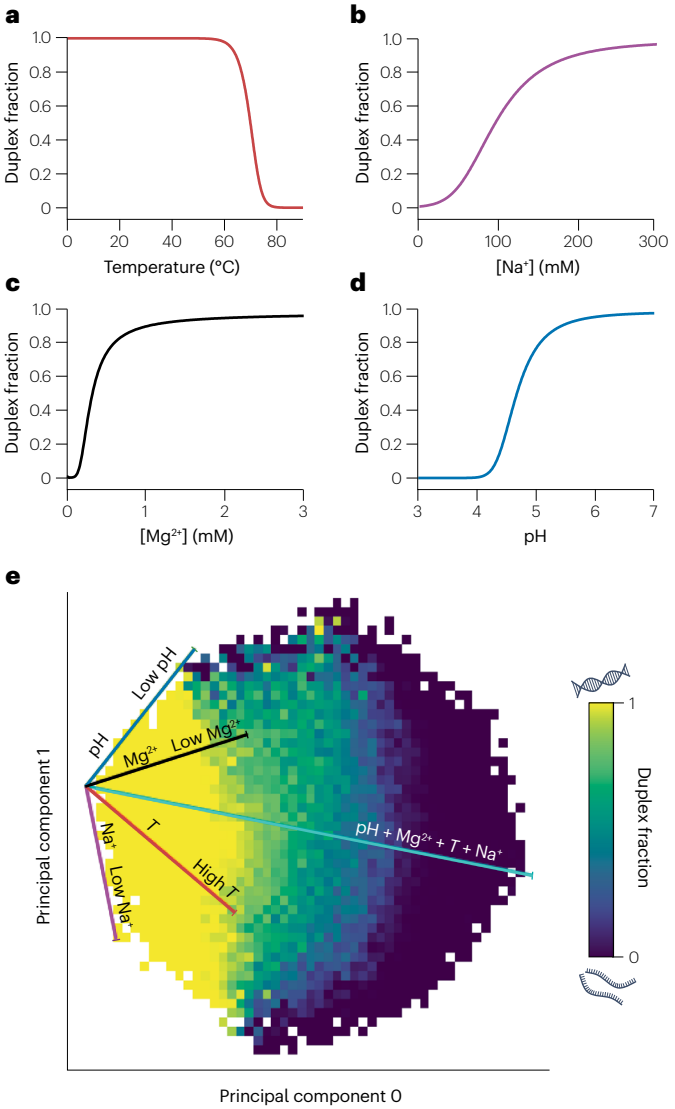

Fig. 2 | The chemico-physical determinants of oligonucleotide stability. **a**–**d**, Melting curves of a 35-nucleotide RNA oligonucleotide (30% GC) as a function of temperature (**a**), concentration of monovalent ions (Na^{+}) (**b**), concentration of bivalent ions (Mg^{2+}) (**c**) and pH (**d**). **e**, Synergistic effects of temperature (T), concentration of Na^{+}, concentration of Mg^{2+} and pH, visualized at reduced dimensionality (2D) via principal component analysis (kernel PCA). Combinations of factors provide enhanced strand separation efficiency. Details are explained in Supplementary Section 1.

from kinetic stalling of mismatching sequences; this sequence selection can strongly reduce the occupied states in the sequence space and promote self-amplification[122].

Sequence-selective UV photodamage

In addition to the impact on prebiotic chemistry discussed above, UV radiation can also photodamage oligonucleotides in a sequence-selective manner[141]. In particular, the excitation energy obtained by the absorption of UV photons can lead to the formation of dimeric lesions from neighbouring pyrimidine nucleotides (TT[142,143], TC[144]) or adenosine nucleotides[145], which can deform the oligomer[146]. Furthermore, the formation of charge transfer states[147,148] in the vicinity of guanosine nucleotides can prevent damage formation, resulting in a strong anticorrelation between the GC content and the susceptibility to UV light[149]. These factors add up to a complex sequence dependence of UV resistance in oligonucleotides[150]. Comparing the UV damage of DNA sequences of 7 nt length (Fig. 3d) indicates that strands that are more resistant to UV damage are enriched in G and C, which also results in a higher melting temperature T_m of the strands. In addition, double-stranded DNA is more resistant to UV damage than single-stranded oligonucleotides[151]. Both effects thus reinforce each other and select more stable, GC-rich DNA duplexes, which are particularly well protected against UV. These observations pose stringently measurable selection and regulation pressures on early sequence pools such as RNA aptamers[152].

Non-canonical sequence evolution

Over the years, many additional approaches have been proposed that use complex chemistry and specific RNA or DNA sequences to trigger processes of replication, recombination and strand separation.

In rolling circle RNA synthesis[153,154], a circular template aids continuous replication by overcoming the problem of the fast reannealing of the complementary strands, which occurs at high concentrations. The model is valid to describe the downstream evolution of polymerase-catalysed replication. It requires the loop to close after the replication in order to offer an exponentially growing replication, which is considered important to maintain information against the exponentially decaying dynamics of degradation. Note that the rather long persistence length of double-stranded RNA of 63 nm (or 250 bases) would probably restrict rolling circle replication under physiological conditions to longer sequences[155].

Another way to provide strand displacement can be through invading RNA strands[156]. Short complementary RNA fragments, derived from degradation, partial replication or non-templated polymerization, are able to attack a double-stranded region and partially open it to allow this short primer to be elongated. This type of strand displacement could allow RNA replication cycles to be completed when the reannealing of complementary strands becomes too fast.

Based on the observation that there are no clear prebiotic mechanisms to discriminate between DNA and RNA, RNA–DNA chimeras have

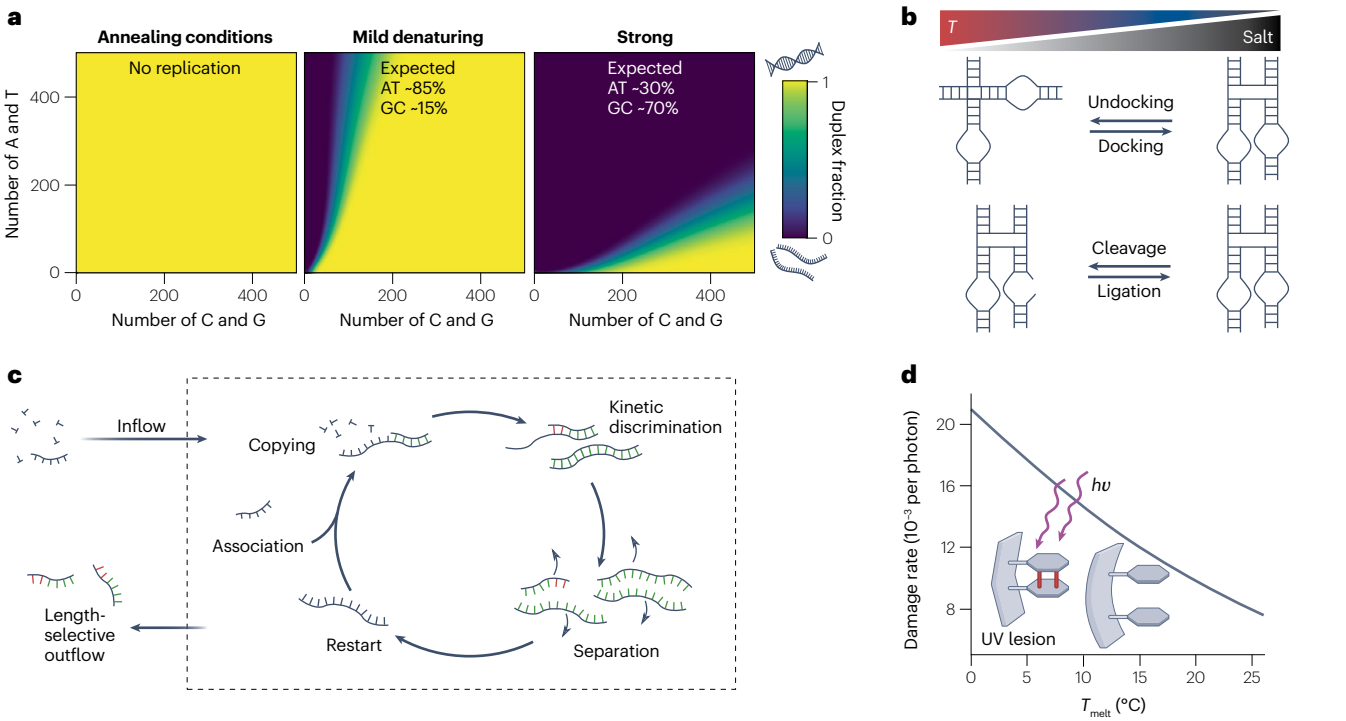

Fig. 3 | Non-equilibrium environments to shape oligonucleotide evolution. **a**, The nucleotide composition of replicating sequences is determined by the chemico-physics of water. Denaturing conditions to form single strands correlate with an increased GC content. Left: annealing conditions (10 °C, pH 7, 300 mM Na^+, 3 mM Mg^{2+}); middle: mild denaturing (65 °C, pH 4.5, 20 mM Na^+, 0.3 mM Mg^{2+}); right: strong denaturing (75 °C, pH 4, 1 mM Na^+, 0.1 mM Mg^{2+}). **b**, The catalytic activity of the hairpin ribozyme is regulated by temperature and concentration of salts, which influence its folding and conformation. **c**, Kinetic selection mechanism for sequence length and error discrimination. The hybridization kinetics of oligonucleotides is also affected by the chemico-physical parameters. **d**, Sequence dependence of ultraviolet damage, indicated as damage rate per photon, for 7-nucleotide single-stranded DNA after 2.5 h of ultraviolet irradiation. Higher melting temperature (T_{melt}) corresponds to stable duplex sequences (GC-rich), which are less susceptible to ultraviolet damage. Details are explained in Supplementary Section 2.

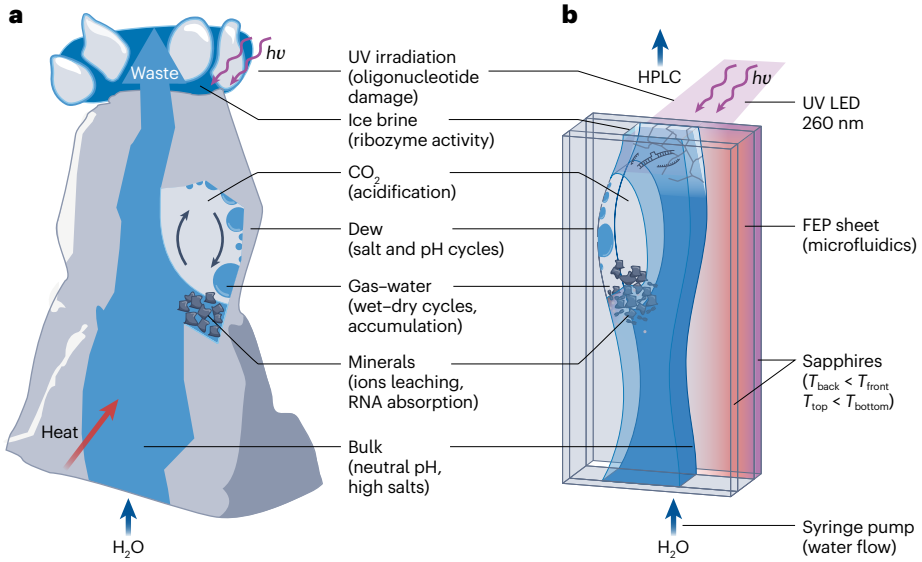

Fig. 4 | Heterogeneous chemico-physical possibilities to drive the origin of life. a, Multiple non-equilibrium features in the same geological setting are prebiotically plausible. Such a complex system would generate unexplored scenarios for the evolution of the first oligonucleotides on the early Earth. **b**, It is experimentally feasible to investigate such a scenario. A microfluidic implementation at the millimetre scale is possible using standard components: electric components for heating and cooling, fluorinated ethylene propylene (FEP) sheets as containers, transparent sapphire windows for imaging, syringe pumps to flow water, and light-emitting diodes (LEDs) for illumination and ultraviolet irradiation. *T*, temperature; HPLC, high-performance liquid chromatography.

been proposed to have played a role during molecular evolution[157–159]. These chimeras have weaker base pairing than the standard duplexes, indicating they would be less affected by the strand separation problem under non-denaturing conditions. Moreover, they add a new possibility to the RNA–DNA–protein world transition model, suggesting the coexistence and co-evolution of RNA and DNA systems during the initial stages. Adding the more stable DNA to the mix for long-term storage of sequences offers many advantages, but it remains to be seen if they are outweighed by the disadvantages associated with the co-replication and co-synthesis of DNA together with RNA. Moreover, it remains unclear what chemico-physical conditions are suited for the replication of a hybrid system containing both RNA and DNA, as they have very different pH, salt and temperature preferences. For example, RNA is more susceptible to hydrolysis than DNA at high temperatures. Moreover, the conditions that enhance RNA catalytic activity[160] (high salts, neutral pH, moderate temperature) are those that better stabilize the DNA double-stranded conformation, posing the problem of how strand separation is to occur. Therefore, salt-poor wet–dry cycling could provide a new perspective[161]; or non-equilibrium environments hosting fluctuating pH, salts and temperatures could offer an interesting environment in which to test the molecular evolution of these types of hybrid systems.

Many of the above ideas to replicate RNA have not yet been tested under geological non-equilibria. Based on experience, we believe that interesting and unexpected dynamics will be found by this connection of geoscience, physics and chemistry. Moreover, the combination of the discussed geophysical features, when coupled with more complex prebiotic chemistries, are expected to offer synergistic results and are now becoming experimentally feasible.

Primordial scenarios and experimental analogues

It is plausible that heterogeneous physico-chemical microscale properties would have been present in close proximity. For example, a porous rock containing water and CO_2 gas inclusions could exist near to a volcanic heat source, bombarded at its top by UV irradiation and undergoing day–night or seasonal freeze–thaw cycles (Fig. 4a). The rock part could vary between micrometres and tens of metres. In such a setting, ions are leached from the minerals to buffer the pH of the bulk and selectively absorb RNA; convection and thermophoresis in the bulk and the gas–water boundaries of the bubble exponentially and selectively accumulate molecules; microscale water cycles in the gas bubble induce pH and salt fluctuations that facilitate strand separation; the UV irradiation induces selective damage to oligonucleotides; and freeze–thaw cycles create ice brines and locally enhance the activity of ribozymes.

This system can be efficiently transferred to a lab experiment and implemented in millimetre-sized microfluidic experiments (Fig. 4b). Heat flow can be provided by differentially heating and cooling the surfaces of the microfluidic chamber, CO_2 gas bubbles can be introduced microfluidically, a continuous liquid flow can be controlled with syringe pumps, realistic geomaterials can be introduced as powdered minerals, and the sample chamber can be locally irradiated with UV-light-emitting diodes. However, the combinatorial space of the prebiotic physico-chemical and environmental conditions is immense, and so the identification of the key factors is not generalizable but depends on each specific scenario. This means that the results are likely to be unpredictable, and mutual, synergistic or inhibitory effects cannot be determined a priori. One possible approach is to systematically study the effects of each non-equilibrium feature separately. Multiple non-equilibrium features can then be merged, once the single effects have been quantified.

Researchers have started to make use of heterogeneous geophysico-chemical conditions to study and enhance prebiotic chemical reactions in such more complex physical boundary conditions[162–165]. One example is the production of nucleobases, ribose, and nucleotide precursors in a comprehensive model of the early Earth atmosphere coupled with an evaporating water pond[166]. The system includes lightning, UV, impact degassing, volcanism, ocean geochemistry and water evaporation. The UV irradiation, lightning and wet–dry cycles in an atmosphere with plausible levels of water, CO_2, H_2 and CH_4 enable a series of complex chemical reactions, from the formation of HCN to sugars and nucleotide precursors. Such a level of complexity would

have not been possible without the interaction of multiple geological features. The interplay between such geological settings and prebiotic chemical reactions is likely to create unpredictable results, with mutual, synergistic or inhibitory effects in a complex reaction network.

Enzymatic replication

Whereas the chemistry for prebiotic RNA replication will probably remain a puzzle for some time, it might be a good exercise to probe the physics of the environment with polymerase or ligase proteins. And indeed, this approach has shown first signs of Darwinian-like evolution. For example, the three-body reaction of templated ligation was observed to create a strong self-selection of initially random sequences, creating a unusually complex sequence space from the random pools[129]. With this self-selection, a sparse exploration of the sequence space becomes possible without having to scan the masses of the Universe that are theoretically necessary to hold all 4^{100} sequences for 100-nucleotide oligomers, as would be required to perform evolution in a very systematic way.

As seen by following the same approach, enzymatic replication at air–water interfaces has interesting strand separation dynamics[51]. Gas bubbles inside thermal gradients, which experimenters normally avoided at great cost in microfluidic experiments, are now hot spots for replicative polymerization. The speed of creating longer strands in these experiments, enzymatically, and protocolled by deep sequencing is compelling. Within a few hours, sequences more than 1,300 nucleotides in length evolved from 50-nucleotide initial templates, adapting to the length-selective non-equilibrium setting at the air–water interface and tuning the AT/GC ratio to fit the conditions of strand separation[51]. Central to this experiment is the local salt and pH cycling at the heated air–water interface, coupled with the capillary flows in the liquid that preferentially accumulate longer oligonucleotides at the gas–water boundaries. Moreover, the replication efficiency of a cold air–water interface[51] or a local heated spot[167] is much higher than ever achieved in bulk polymerase chain reaction (PCR) or systematic evolution of ligands by exponential enrichment (SELEX) experiments[168]. PCR run under non-equilibria on a sequence mix can amplify specific sequences locally in micrometre-sized spots, without depleting the bulk's reagents, and reaching extremely high amplification factors. However, standard bulk amplification typically runs out of resources after 20–30 cycles.

Prebiotic polymerization

A widely investigated topic in the origin of life field is the prebiotic polymerization of RNA. To form RNA from its nucleotides — or peptides from amino acids — water must be removed[169,170]. For nucleotides, modern biology uses triphosphates as an activation group to drive this reaction. When it comes to prebiotic scenarios, the approaches differ on whether to start with a strong activation group and drive the reaction mainly chemically[171–173] or to use a weak activation group and mainly put the burden on the physical non-equilibria, for example by temporarily using dry or semi-dry conditions and active accumulation mechanisms. An example of the latter approach is the polymerization of 2′,3′-cyclic monophosphate nucleotides at elevated pH. Under conditions of temporary drying at a heated air–water interface, RNA oligomers form[161]. One could also set templated replication reactions to operate in similar semi-dry conditions and use the salt-poor wet conditions to separate the RNA strands. This inverted mechanism could offer a number of new perspectives for replicative systems, including longer lifetimes of RNA in the predominantly dry state.

Outlook

In our view, future generations of experiments will include an increasing number of the non-equilibria discussed above, with many more to be discovered. So we think that much can be expected from the above-mentioned non-equilibrium possibilities that geo-environments offer at the microscale (Fig. 4).

We have discussed the multiple ways in which environmental factors are not mere boundary conditions, but instead play an active role in driving and shaping the molecular evolution of DNA and RNA. Based on the positive cooperative effects seen in experiments using this combination of origin of life chemistry with the geoscience of the early Earth in the past, it is likely that scenarios will emerge that can make prebiotic processes much more plausible. The purpose of this Perspective is to encourage the use of non-equilibria in origin of life experiments. We have discussed and demonstrated many intrinsic advantages of combining the physics of selection and strand separation with the chemistry of replication in a natural localized setting. Apart from these biotechnological advantages, we argue that the merging of physics and chemistry is the best route to understand, through lab experiments, that the emergence of life might not have been as rare and implausible as is commonly thought. Many more interesting molecular machines recreating the emergence of life may be found at the crossroads of replication chemistry and non-equilibrium physics. The future of cross-disciplinary experiments has only just begun in this field.

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Competing interests

The authors declare no competing interests.

Additional information

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