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Is there a common chemical model for life in the universe?

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A review of organic chemistry suggests that life, a chemical system capable of Darwinian evolution, may exist in a wide range of environments. These include non-aqueous solvent systems at low temperatures, or even supercritical dihydrogen–helium mixtures. The only absolute requirements may be a thermodynamic disequilibrium and temperatures consistent with chemical bonding. A solvent system, availability of elements such as carbon, hydrogen, oxygen and nitrogen, certain thermodynamic features of metabolic pathways, and the opportunity for isolation, may also define habitable environments. If we constrain life to water, more specific criteria can be proposed, including soluble metabolites, genetic materials with repeating charges, and a well defined temperature range.

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Introduction

A simple 'We don't know' is often the best answer for some questions, perhaps even for the title question, suggested by this issue's editors of *Current Opinion in Chemical Biology*. Because we have direct knowledge of life only on Earth, and as known life on Earth descended from a single ancestor, we have only one data point from which to extrapolate statements about the chemistry of life generally. Until life is encountered elsewhere, or aliens contact us, we will not have an independent second dataset. We may not even then, if the alien life itself shares an ancestor with life on Earth.

We can, of course, conceive of alternative chemical solutions to specific challenges presented to living systems [1••]. We can then test their plausibility by synthesizing, in the laboratory, unnatural organic molecules that represent the alternatives, and seeing if they behave suitably. Some of these alternatives come from simple 'Why?' or 'Why not?' questions. For example: why are 20 standard amino acids used in Terran proteins? Experiments with unnatural amino acids (using the natural ribosome to incorporate them into proteins) have expanded the amino acid repertoire of proteins [2–7]. These experiments find no reason to exclude alternative sets of amino acids from hypothetical proteins in hypothetical alien life forms.

Similar questions can be asked about DNA. For example, why does Terran genetics use ribose and deoxyribose? Why not glycerol, a hexose or a tetrose? Again, synthetic chemists have made DNA analogs using each, and studied their behavior [8–10]. These studies underlie a rational discussion of the etiology and design of DNA.

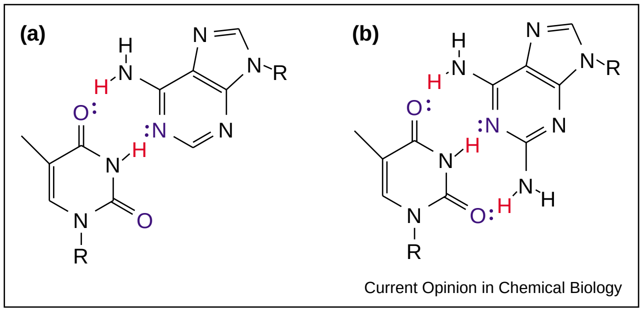
Some questions reach farther. Is a three-biopolymer system (DNA–RNA–proteins) essential for life? Why not two biopolymers, or just one? Is water necessary? Is carbon essential? Why not silicon? Here, direct experiments are more difficult to conceive; few have actually been attempted.

Nevertheless, a discussion of 'Why?' and 'Why not?' questions posed by Terran biochemistry can start with a trialectic of three classes of explanations [11]. The first class is functional, and hypothesizes that a chemical structure is found in life because it is the best solution to a particular biological problem. Under the Darwinian paradigm, 'best' means 'confers most fitness', or (at least) more fitness than unused alternatives. This explanation implies that the living system had access to alternative solutions, individuals chose among these, and those who chose poorly failed to survive.

The second class of explanation is historical. The universe of chemical possibilities is huge. For example, the number of different proteins 100 amino acids long, built from combinations of the natural 20 amino acids, is larger than the number of atoms in the cosmos. Life on Earth certainly did not have time to sample all possible sequences to find the best. What exists in modern Terran life must therefore reflect some contingencies, chance events in history that led to one choice over another, whether or not the choice was optimal.

Explanations of this class are difficult to evaluate because we know little about the hypersurface relating the sequence of a protein to its ability to confer fitness [11]. If that hypersurface is relatively smooth, then optimal solutions might be reached via Darwinian search processes, regardless of where contingencies began the search. If the surface is rugged, however, then many structures of Terran life are locally optimal, and suboptimal with

Figure 1



The thymine–adenine T•A base pair **(a)** is joined by two hydrogen bonds. Why is aminoadenine **(b)** not used instead of adenine, to give a stronger base pair joined by three hydrogen bonds?

respect to the universe of possibilities. Darwinian mechanisms cannot easily find a globally optimal solution on a rugged hypersurface.

The third class draws on the concept of vestigiality. Here, it is recognized that some features of contemporary Terran biochemistry may have emerged via selective pressures that no longer exist. The contemporary feature may therefore not represent optimization for the modern world, but rather be a vestige of optimization in an ancient world. Such models explain, for example, the human appendix, and many of the chemical details found in modern life (such as the RNA component of many cofactors).

These three classes of explanations are illustrated by possible answers to a question asked by many biochemistry students: why does DNA use adenine, which forms only two hydrogen bonds with thymidine? Aminoadenine gives stronger pairing [12••] (Figure 1). A functional explanation might argue that genomes are optimal when they have both a weak (A•T) and strong (G•C) pair. A historical explanation suggests that adenine was arbitrarily chosen over other available candidates by a contingent accident, and conserved because it was too difficult to later replace without losing fitness. A vestigiality explanation holds that adenine, not aminoadenine, is made prebiotically from ammonium cyanide [13]. Here, adenine's prebiotic availability made it better for starting life, even though aminoadenine might be preferable now.

It is useful to construct these alternative explanations as hypotheses, even if no experiments can presently test them. The act of constructing the trialectic allows scientists to appreciate how accessible alternative explanations are. This, in turn, provides a level of discipline to a discussion where the 'We don't know' answer is probably most appropriate. This article reviews lightly some of the chemical constraints on life, and some possible chemistries of alternative life forms.

What is Life?

To decide whether life has a common chemical plan, we must decide what life is [14••,15••]. A panel assembled by NASA in 1994 was one of many groups to ponder this question. The panel defined life as a 'chemical system capable of Darwinian evolution' [16]. This definition, which follows an earlier definition by Sagan [17], will be used here.

This definition contrasts with many others that have been proposed, and avoids many of their pitfalls. For example, some definitions of life confuse 'life' with the concept of being 'alive'. Thus, asking if an entity can move, eat, metabolize or reproduce might ask whether it is alive. But an individual male rabbit (for example) that is alive cannot (alone) support Darwinian evolution, and therefore is not 'life' [18].

Further, many efforts to define 'life' fall afoul of the fact that no non-trivial term can be defined to philosophical completeness [19]. The general difficulty of defining terms, theoretically or operationally, was one of the discoveries of 20th century philosophy, and is not unique to the definition of 'life'. It is impossible, for example, to define 'water' in a complete way. We can say that water is 'dihydrogen oxide', but are we speaking of a water molecule, water as a substance, or water operationally? And what is 'hydrogen'? Any effort to deal with the definition of life at this level encounters analogous questions, which can easily be paralyzing.

The phrase 'Darwinian evolution' carries baggage from 150 years of discussion and elaboration. It makes specific reference to a process that involves a molecular system (DNA on Earth) that is replicated imperfectly, where the imperfections are themselves heritable. Therefore, Darwinian evolution implies more than reproduction, a trait that ranks high in many definitions of life.

The panel's definition also avoids confusion from many non-living systems that reproduce themselves. For example, a crystal of sodium chlorate can be powdered and used to seed the growth of other crystals [20]; the crystal thereby reproduces. Features of the crystal, such as its chirality, can be passed to descendants [20]. The replication is imperfect; a real crystal of sodium chlorate contains defects. To specify all of the defects would require enormous information, easily the amount of information in the human genome. But the information in these defects is not itself heritable. Therefore, the crystal of sodium chlorate cannot support Darwinian evolution. Therefore, a sodium chlorate system is not life [21•].

The NASA panel's definition of life is interesting for other reasons. First, it provides information about what forms of life were believed to be possible, not just conceivable. As fans of Star Trek know, forms of life that are *not* chemical systems capable of Darwinian evolution are easily conceivable. Besides aliens resembling Hollywood actors with prostheses, the Enterprise has encountered conceptual aliens that do not fit the panel's definition. The nanites that infected the Enterprise computer in Episode 50 of Star Trek: The Next Generation ('Evolution') are informational in essence; their Darwinian evolution is not tied to an informational molecule, like DNA (although they require a chemical matrix to survive). The Crystalline Entity of Episodes 18 ('Home Soil') and 104 ('Silicon Avatar') appears to be chemical, but not obviously Darwinian. The Calamarain (Episode 51: 'Déjà Q') are a conceptual life form that is purely energy, not evidently requiring matter. And Q (Episode 1: 'Encounter at Farpoint') appears to be neither matter nor energy, flitting instead in and out of the Continuum without the apparent need of either.

If we were to encounter any of these other conjectural entities during a real, not conceptual, trek through the stars, we would be forced to concede that they represent living systems. We would be obligated to change our definition of life. We do not change it now simply because we do not believe that the weirder life conceived in the Star Trek scripting room is possible outside of that room.

Nanites and the fictional android Data are examples of artificial life. We do not doubt that androids can be created, including androids who (note the pronoun) desire to be human. We do not, however, believe that Data could have arisen spontaneously, without a creator that had already emerged by Darwinian process (as is indeed the case with Data). Hence, we might regard Data as a biosignature, or even agree that he is alive, even if we do not regard him as a form of life. For the same reason, a computer holding nanites would be evidence that a life form existed to create it; the computer is a biosignature, and the nanites are an artificial life form, something requiring natural life to emerge.

The basic requirements for Life

The NASA panel's definition is also useful because it makes direct reference to chemical principles, which we understand well from Terran science. Chemical principles generate a hierarchy of requirements needed for life under the definition. These have a good chance of being common for life in the cosmos, at least the life falling within the definition. We list them from the most stringent (and most likely to be universal) to the less stringent.

Thermodynamic disequilibrium

To the extent that Darwinian evolution is a progressive process, and to the extent that life actually does something, life requires an environment that is not at thermodynamic equilibrium. This statement is almost certainly true for all life, including artificial life. The statement does not imply a universal chemical metabolism, even on Earth. Wherever a thermodynamic disequilibrium exists on Earth, organisms appear to have adapted a different metabolism to exploit it. But these organisms all require the disequilibrium.

The requirement for an environment that is at disequilibrium is often paraphrased as a requirement for an 'energy source' or 'high energy compounds'. While technically true, this locution can create problems. For example, de Duve speaks of life emerging in acidic environments at high temperatures, because 'energy rich' thioesters form spontaneously in such environments [22]. Indeed they do. But in that environment, thioesters are not 'high energy' (they form spontaneously). Likewise, in an environment poor in water but rich in ADP and inorganic phosphate, the formation of ATP is spontaneous, and ATP has a lower 'energy' than ADP and inorganic phosphate.

Thermodynamic disequilibrium is easy to find in the cosmos. Almost any environment in the vicinity of a nuclear fusion reaction, such as that occurring in our Sun, will not be at thermodynamic equilibrium. Indeed, unshielded exposure to the energy efflux of the Sun creates a problem of having too much energy, as those with sunburn appreciate.

For most of human history, a star was regarded as the *only* way to obtain thermodynamic non-equilibrium (Genesis 1.3 and the Popol Vuh, for example). Robert Heinlein suggested that the ultimate punishment from an intergalactic court is to deprive a miscreant planet of its sun [23]. In this view, a universal feature of life is a food chain that begins with an autotrophic organism that converts photons from a star into chemical disequilibrium.

But other disequilibria are available within planets. For example, heavier atomic nuclei, left from a supernova, are not at thermodynamic equilibrium. Decay of these nuclei is a powerful source of planetary not-at-equilibrium environments. Radioactive decay drives tectonics and volcanism on Earth. These create non-equilibrium environment in many areas [24], such as black smokers on the ocean floor [25]. The consequent energetic disequilibrium supports life despite the absence of direct solar energy.

Recognizing that geothermal energy is easily placed at the bottom of a food chain, Stevenson noted that small, Earth-like planets that support life from geothermal energy might be ejected from forming solar systems [26]. They could then travel the galaxy, carrying life completely independent of a star. Depending on the frequency of these ejections, such planets might hold the vast majority of life in our galaxy, all living without a sun on the decay of radioactive nuclei left over from past supernova explosions.

Bonding

A weaker constraint on life requires that it be based on covalent bonds. The concept is imperfect; the distinction between covalent and non-covalent bonding itself reflects the language of organisms living at 310K. There is, in fact, a range of chemical bonds, from the very strong, surviving for significant times even at temperatures as high as 1000K, to the very weak, existing only near absolute zero.

Terran life uses various types of bonding. The carbon–carbon covalent bond, typically 400 kJ mol^{-1} with respect to homolytic fragmentation, is the strongest of these. A hydrogen bond is worth only 20 kJ mol^{-1}. It is nevertheless strong enough to be important in Terran life at 273–373 K. The hydrogen bond gives water its most distinctive physical properties, including its high boiling point, the large range in temperature over which it is a liquid, and its increase in volume upon freezing. In addition, the hydrogen bond is largely responsible for the hydrophobic effect, and the fact that water and oil form separate phases at 310 K.

Most of the universe does not lie between 273 and 373 K, however, the range where covalent bonds are necessary and hydrogen bonds are useful. At lower temperatures, biochemistry may well be dominated by non-covalent bonds. The bonding that supports information transfer needed for Darwinian evolution might universally require bonding sufficiently strong at the ambient temperature to be stable for some appropriate time. In water at 273–373 K, a combination of covalent bonding, hydrogen bonding and the hydrophobic effect meets this requirement. In different solvents or temperatures, different bonding may be better suited.

Isolation within the environment

Selfishness is essential to Darwinian evolution. A Darwinian cycle can proceed only if it replicates itself in preference to others. An enormous literature discusses isolation in the context of parasitism and altruism [27]. Much of the recent literature on molecular systems to achieve isolation focus on liposomes and other membrane-like structures that resemble lipid bilayers found throughout Terran life [28–31]. An interesting result from the Szostak laboratory connects mineralogy to liposome formation [32]. This type of compartmentalization is based on liquid/liquid immiscibility and the hydrophobic effect. This need not be the only way to achieve isolation, of course. Isolation can be achieved on a two-dimensional surface, where living system has a fractile 2+ dimensionality [33].

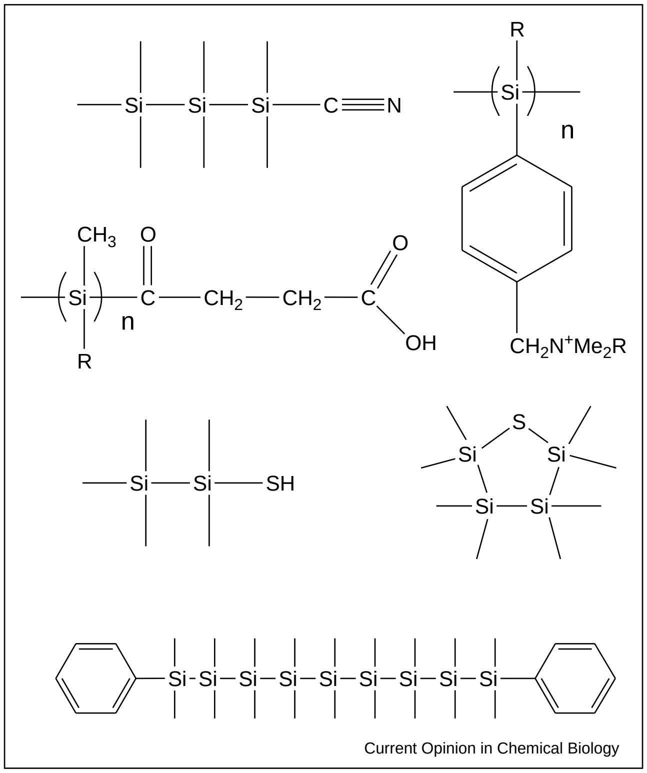
Carbon-like scaffolding

The stability of the carbon–carbon bond at 273–373 K has made it the first choice element to scaffold biomolecules. Even in Terran biochemistry, other elements are required. Hydrogen is needed for many reasons; at the very least, it terminates C–C chains. Heteroatoms (atoms that are neither carbon nor hydrogen) determine the reactivity of carbon-scaffolded biomolecules. In Terran life, these are oxygen, nitrogen and, to a lesser extent, sulfur, phosphorus, selenium, and an occasional halogen.

While carbon is widely regarded as the only scaffolding element [34,35], silicon is a frequently mentioned second choice (see the original Star Trek Episode 26, 'The Devil in the Dark'). Like carbon, silicon can form four bonds. The silicon–silicon bond is weaker than the carbon–carbon bond [36], but not excessively (typically ca. 300 kJ mol^{-1}, compared with 400 kJ mol^{-1} for a typical carbon–carbon bond).

Many compounds built on a scaffolding of silicon–silicon bonds have in fact been made. Oligosilanes having up to 26 consecutive Si–Si bonds are known; these support a variety of functionalized and non-functionalized side chains (Figure 2). These structures resemble those from Terran membranes. These have been targets of chemists because of their ability to form sigma-conjugated polysilanes [37]. The electronic properties of these compounds are similar to those of the carbon-containing pi-conjugated system.

Figure 2



Structures of some synthetic polysilanes that have been described in the literature. There is little question that polymeric diversity is possible using silicon, rather than carbon, as a scaffolding.

Oligosilanes generally have alkyl side chains. These are generally soluble in non-polar solvents. Shorter polysilanes are known containing reactive ends (cyanide, hydroxyl or phenol) that allow further modifications and solubility in polar solvents. For example, phenolic oligosilanes dissolve in alcohol, ether acetone and slightly alkaline water. Oligosilanes carrying carboxylic acid groups are soluble in water [38,39]. Amphiphilic oligosilanes self-aggregate in water, creating vesicles and micelles [40]. Oligosilanes can also be chiral (*pace* the statement on the NASA astrobiology web http://nai.arc.nasa.gov/astrobio/feat_questions/silicon_life.cfm).

The silicon–oxygen–silicon (silicone) unit also makes scaffolds, found today in superballs and breast implants. Again, silicon and oxygen alone cannot generate particularly interesting chemistry. For this, the Si–O– units must have side chains that include carbon, hydrogen, nitrogen, oxygen and other heteroatoms. The life would nevertheless be called silicon-based, just as we call Terran life carbon-based, even though it depends on other atoms as much as on carbon.

The reactivities of silicon and carbon differ in some notable ways. First, its position in the third row of the periodic table means that silicon carries low energy *d* orbitals. Therefore, the associate mechanism for reaction at silicon, where an incoming nucleophile forms a bond before a leaving group leaves, is available to silicon, but not to carbon. This makes many compounds containing silicon more reactive than their carbon analogs. Whether this difference in reactivity is advantageous or disadvantageous depends on perspective. The greater reactivity of silicon compared with carbon may be an advantage in cold environments [1].

The weaker criterion is that life requires scaffolding based on carbon *or* silicon. There is no third choice if covalent scaffolding is viewed as a requirement. Weirder forms of life based on ionic bonding, or metallic bonding, are conceivable in principle, but not otherwise at this time. Nevertheless, it is instructive to review the writings of Cairns-Smith and Hartman [41,42], who have considered issues relating to information transfer in systems that are neither carbon-based nor dependent on covalent bonding.

Energetic patterns in metabolism

For a metabolic sequence to convert substrates into products, the two must be in disequilibrium. When they are not, the overall reaction must be coupled to another reaction where substrates and products are at disequilibrium. This is frequently phrased by textbooks as a requirement that a metabolic pathway must be energetically downhill, or coupled to an energy source.

Many Terran metabolic multistep pathways are run close to equilibrium for internal steps (Figure 3), with the first step being energetically downhill, and a site of regulation. Required, however, is that the last step be energetically downhill, thereby pulling the reaction to completion [43]. This feature may be universal in metabolic pathways, simply because it exploits most economically a surrounding chemical disequilibrium. Having large free energy drops at every step in a pathway is wasteful.

This characteristic energetic relationship between a set of compounds that are intermediates in an evolved metabolism may be a universal biosignature. If an inventory of the small molecules in a suspected living system (on Saturn's largest moon, Titan, for example) reveals this characteristic energetic relationship, this may be evidence of Darwinian evolution acting to create an optimal metabolism.

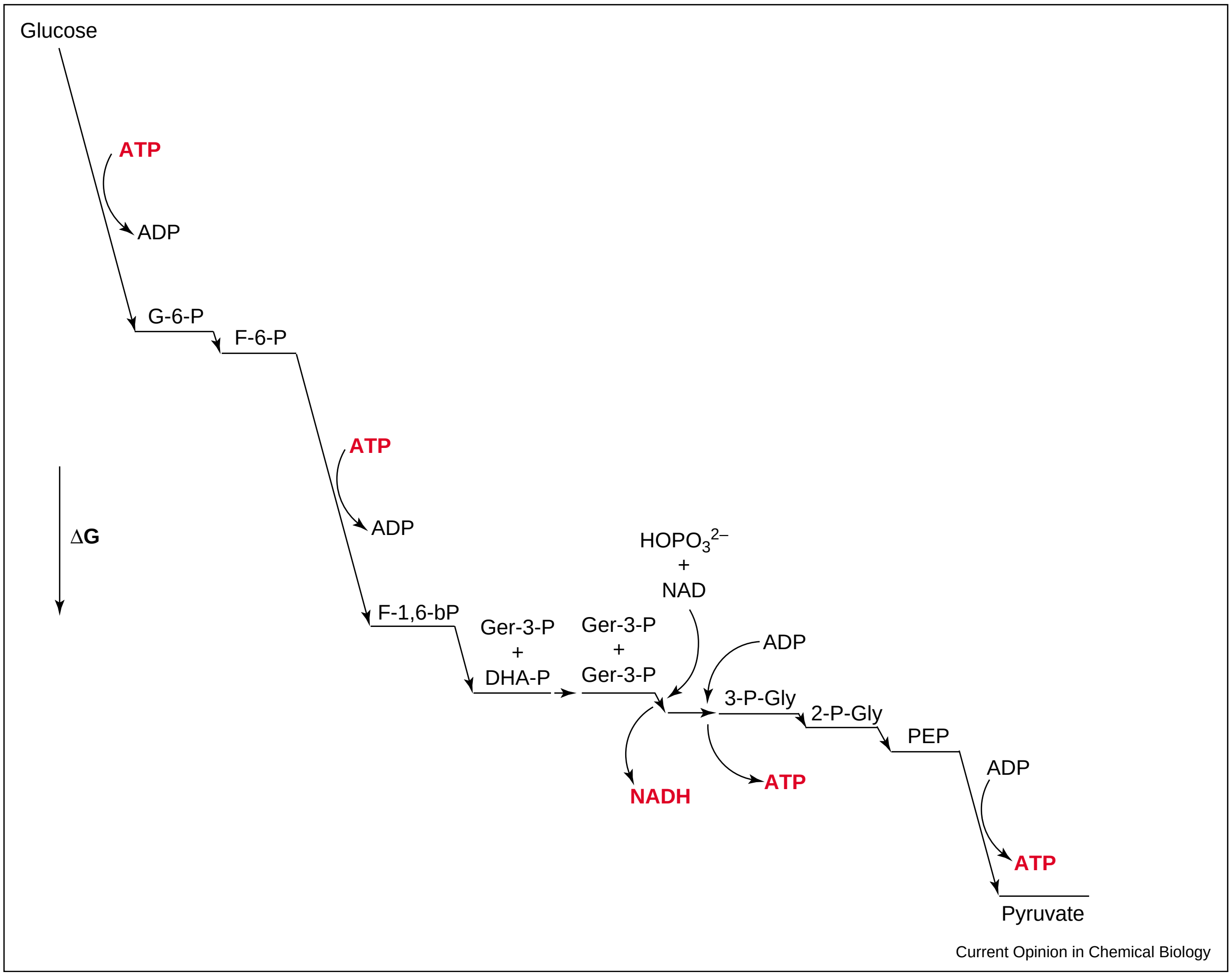
The solvent

A liquid phase facilitates chemical reactions, something known empirically for centuries. As a solvent, a liquid allows dissolved reactants to encounter each other at rates higher than the rates of encounter between species in a solid. Chemical reactions can take place in the gas and solid phases as well, of course. But each of these has disadvantages relative to the liquid phase.

In the gas phase, chemistry is limited to molecules that are sufficiently volatile to deliver adequate amounts of material to the gas phase at moderate temperatures, and/or to molecules sufficiently stable to survive higher temperatures where vapor pressures are higher. Obviously, if volume is abundant, pressures are low, and time scales are long, even low concentrations of biomolecules might support a biosystem in the gas phase. It is even conceivable that in the vacuum of interstellar space, life exists through molecules at high dilution reacting in the gas phase. The possible disadvantage of this is, of course, the difficulties in holding together the components of the interstellar life form. One advantage would be that it need not be encumbered by the lifetime of a planetary system. Indeed, one can imagine life in the gas phase associated with a galaxy and its energy flux for nearly the age of the universe.

Likewise, species can diffuse through solids to give chemical reactions [44]. Solid-phase diffusion is slow, however. Nevertheless, given cosmic lengths of time, and the input of energy via high energy particles, a biochemistry able to support Darwinian evolution can be conceived [45]. A weird life form might reside in solids of the Oort cloud (a large sphere of cosmic debris surrounding the solar system, and the origin of comets) living in deeply frozen water, obtaining energy occasionally from the trail of free radicals left behind by ionizing radiation, and carrying out only a few metabolic transformations per millennium.

Figure 3



Physiological free energy diagram for the glycolysis reaction for one of the two Ger-3-P generated. G, glucose; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-bP, fructose-1,6-bisphosphate; Ger-3-P, glyceraldehyde-3-phosphate; DHA-P, dihydroxyacetone-P; 1,3-bP-Gly,1,3-bisphosphoglycerate; 3-P-Gly, 3-phosphoglycerate; 2-P-Gly, 2-phosphoglycerate; PEP, phosphoenolpyruvate. Vertical height is proportional to the change in free energy (ΔG).

Solvents like water, but not water

Liquid ammonia is a possible solvent for life, an alternative to water. Indeed, water and ammonia are analogous. Ammonia, like water, dissolves many organic compounds. Preparative organic reactions are done in ammonia in the laboratory. Ammonia, like water, is liquid over a wide range of temperatures (195–240 K at 1 atm). The liquid range is even broader at higher pressure. For example, at 60 atm ammonia is liquid at 196–371 K. Further, liquid ammonia may be abundant in the solar system. It exists, for example, in the clouds of Jupiter.

The weaker hydrogen-bonding potential of ammonia is often considered undesirable in some discussions of ammonia as a biosolvent. Ammonia has three hydrogen bond donors and only one hydrogen-bonding acceptor, whereas water has two of each. This imbalance is used to explain the lower boiling point of ammonia compared with water.

The increased ability of ammonia to dissolve hydrophobic organic molecules (again compared with water) suggests an increased difficulty in using the hydrophobic effect to generate compartmentalization in ammonia, relative to water. This, in turn, implies that liposomes, a compartment that works in water, generally will not work in liquid ammonia.

Hydrophobic phase separation is possible in ammonia, however, albeit at lower temperatures. For example, Brunner reported that liquid ammonia and hydrocarbons form two phases, where the hydrocarbon chain contains 1–36 CH_2 units [46]. Different hydrocarbons become miscible with ammonia at different temperatures and pressures. Thus, phase separation useful for isolation would be conceivable in liquid ammonia at temperatures well below its boiling point at standard pressures.

The greater basicity of liquid ammonia must also be considered. The acid and base in water are H_3O^+ and HO^-. In ammonia, NH_4^+ and NH_2^- are the acid and base, respectively. H_3O^+, with a pK_a of −1.7, is ca. 11 orders of magnitude stronger an acid than NH_4^+, with a pK_a of 9.2 (in water). Likewise, NH_2^- is about 15 orders of magnitude stronger as a base than HO^-.

Ammonia would not support the chemistry found in Terran life, of course. Terran life exploits compounds containing the C=O carbonyl unit. In ammonia, carbonyl compounds are converted to compounds containing the corresponding C=N unit. Nevertheless, hypothetical reactions that exploit a C=N unit in ammonia can be proposed in analogy to the metabolic biochemistry that exploits the C=O unit in Terran metabolism in water (Figure 4) [47]. Given this adjustment, metabolism in liquid ammonia is easily conceivable.

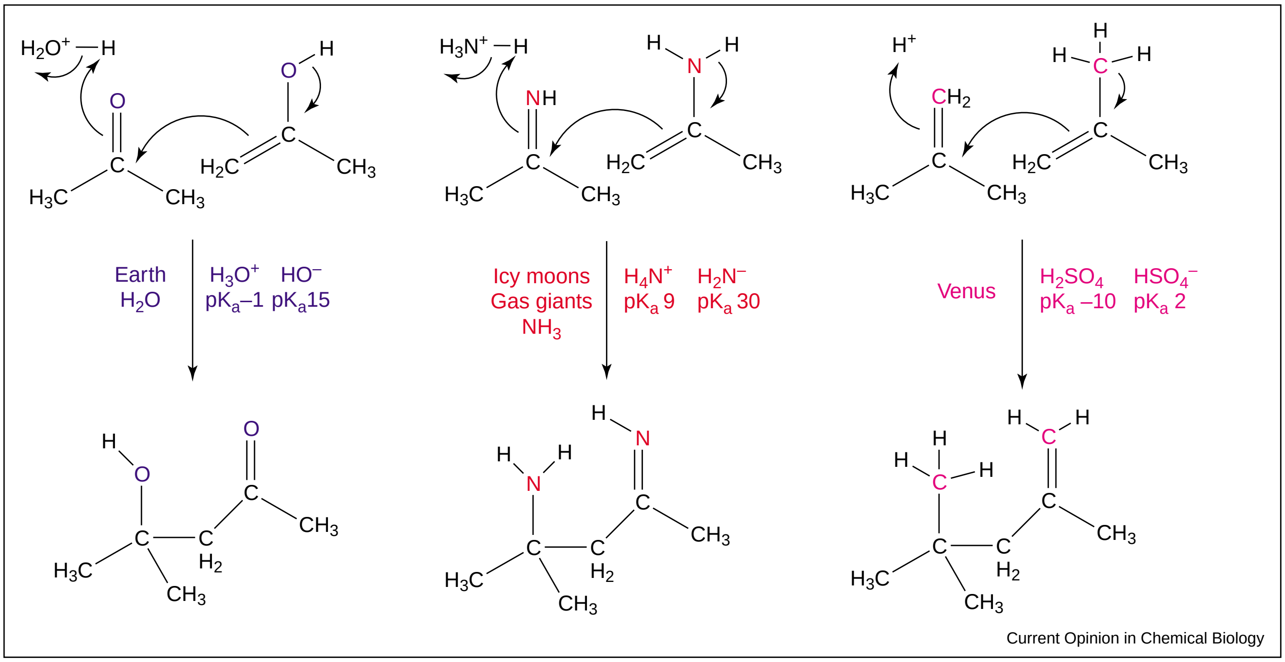
Ammonia is not the only polar solvent that might serve as an alternative to water. For example, sulfuric acid is a reasonably good solvent that supports chemical reactivity [48]. Sulfuric acid is known above Venus [49]. Here, three cloud layers at 40–70 km are composed mostly of aerosols of sulfuric acid, ca. 80% in the upper layer and 98% in the lower layer [50•]. The temperature (ca. 310 K at ca. 50 km altitude, at ca. 1.5 atm) is consistent with stable carbon–carbon covalent bonds.

Many authors have discussed the possibility of life on Venus in its acidic environment [51,52], replacing earlier views that Venus might be covered by swamps [53], or by hot seas at the poles [54]. The surface temperature of Venus is approximately 740 K. Sagan and Morowitz even considered organisms that float above the hot surface using hydrogen 'float bladders' [55] analogous to those found in Terran aquatic organisms [56]. Schulze-Makuch argued for sample return from the Venusian atmosphere to address the possibility of life there [57].

Metabolic hypotheses are not in short supply for the hypothetical life in acidic aerosols. In strong acid, the C=O bond is reactive as a base, and can support a metabolism as an analog of the C=O unit (Figure 4). This type of chemical reactivity is exemplified in some Terran biochemistry. For example, acid-based reactions of the C=O unit are used by plants when they synthesize fragrant molecules [58,59]. Nor are sources of energy in short supply in the Venusian atmosphere. For example, a Venusian metabolism might exploit the relatively high flux of ultraviolet radiation in the Venusian clouds [60].

Formamide is a third solvent of biological interest. Formamide, formed by the reaction of hydrogen cyanide

Figure 4



Different functional groups, but analogous mechanisms, could be used to form new carbon–carbon bonds in different solvents. In water, the C=O unit would provide the necessary reactivity. In ammonia, the C=N unit would provide the necessary reactivity. In sulfuric acid, the C=O unit is sufficient to provide the necessary reactivity.

with water, is polar like water. In formamide, however, many species that are thermodynamically unstable in water with respect to hydrolysis, are spontaneously synthesized. This includes ATP (from ADP and inorganic phosphate), nucleosides (from ribose borates [61] and nucleobases), peptides (from amino acids), and even oligoribonucleotides [62–64]. Formamide is itself hydrolyzed, meaning that it persists only in a relatively dry environment, such as a desert. As desert environments have recently been proposed as being potential sites for the prebiotic synthesis of ribose [61], they may hold formamide as well.

Hydrocarbons: Non-polar solvents for biology?

There is no need to focus on polar solvents, like water, when considering possible habitats for life. Hydrocarbons, ranging from the smallest (methane) to higher homologs (ethane, propane, butane etc.) are abundant in the solar system.

Methane, ethane, propane, butane, pentane and hexane have boiling points of ca. 109, 184, 231, 273, 309 and 349 K, respectively, at standard pressure. Thus, oceans of ethane may exist on Titan. At a mean surface temperature estimated to be 95 K, methane (which freezes at 90 K) would be liquid, implying that oceans of methane could cover the surface of Titan.

Many discussions of life on Titan have considered the possibility that water, normally frozen at the ambient temperature, might remain liquid following heating by impacts [65]. Life in this aqueous environment would be subject to the same constraints and opportunities as life in water. Water droplets in hydrocarbon solvents are, in addition, convenient cellular compartments for evolution, as Tawfik and Griffiths have shown in the laboratory [66]. An emulsion of water droplets in oil is obtainable by simple shaking. This could easily be a model for how life on Titan achieves Darwinian isolation.

But why not use the hydrocarbons that are naturally liquid on Titan as a solvent for life directly? Broad empirical experience shows that organic reactivity in hydrocarbon solvents is no less versatile than in water. Indeed, many Terran enzymes are believed to catalyze reactions by having an active site that is not water-like.

Further, hydrogen bonding is difficult to use in the assembly of supramolecular structures in water. In ethane as a solvent, a hypothetical form of life would be able to use hydrogen bonding more; these would have the strength appropriate for the low temperature. Further, hydrocarbons with polar groups can be hydrocarbon-phobic; acetonitrile and hexane, for example, form two phases. One can conceive of liquid/liquid phase separation in bulk hydrocarbon that could achieve Darwinian isolation.

The reactivity of water means that it destroys hydrolytically unstable organic species. Thus, a hypothetical form of life living in a Titan hydrocarbon ocean would not need to worry as much about the hydrolytic deamination of its nucleobases, and would be able to guide reactivity more easily than life in water.

This is understood by preparative organic chemists, who prefer non-aqueous solvents to water as media for running organic reactions in the laboratory. For example, in a recent issue of the *Journal of Organic Chemistry*, chemists used a solvent other than water to run their reactions over 80% of the time. Chemists avoid water as a solvent because it itself is reactive, presenting both a nucleophilic oxygen and an acidic hydrogen at 55 molar concentrations. Thus, in many senses, hydrocarbon solvents are better than water for managing complex organic chemical reactivity.

Thus, as an environment, Titan certainly meets all of the stringent criteria outlined above for life. Titan is not at thermodynamic equilibrium. It has abundant carbon-containing molecules and heteroatoms. Titan's temperature is low enough to permit a wide range of bonding, covalent and non-covalent. Titan undoubtedly offers other resources believed to be useful for catalysis necessary for life, including metals and surfaces.

This makes inescapable the conclusion that if life is an intrinsic property of chemical reactivity, life should exist on Titan. Indeed, for life *not* to exist on Titan, we would have to argue that life is *not* an intrinsic property of the reactivity of carbon-containing molecules under conditions where they are stable. Rather, we would need to believe that either life is scarce in these conditions, or that there is something special, and better, about the environment that Earth presents (including its water).

Solvents that are not water and not hydrocarbon

The most abundant compound in the solar system is dihydrogen, the principal component (86%) of the upper regions of the gas giants, Jupiter, Saturn, Uranus and Neptune. The other principal component of the outer regions of the giant planets is helium (0.14%). Minor components, including methane (2×10^{-3}%), water (6×10^{-4}%), ammonia (2.5×10^{-4}%), and hydrogen sulfide (7×10^{-5}%) make up the rest.

But is dihydrogen a liquid? The physical properties of a substance are described by a phase diagram that relates the state of a material (solid of various types, liquid or gas) to temperature and pressure. A line typically extends across the phase diagram. Above this line, the substance is a gas; below the line, the substance is a liquid. Typically, however, the line ends at a critical point. Above the critical point, the substance is a supercritical fluid, neither liquid nor gas. Table 1 shows the critical temperatures and pressures for some substances common in the solar system.

Table 1

Critical temperature and pressure for selected substances.

Liquid	Critical temperature (K)	Critical pressure (atm)
Hydrogen	33.3	12.8
Neon	44.4	26.3
Nitrogen	126	33.5
Argon	151	48.5
Methane	191	45.8
Ethane	305	48.2
Carbon dioxide	305	72.9
Ammonia	406	112
Water	647	218

The properties of supercritical fluids are generally different than those of the regular fluids. For example, supercritical water is relatively non-polar and acidic. Further, the properties of a supercritical fluid, such as its density and viscosity, change dramatically with changing pressure and temperature as the critical point is approached.

The changing solvation properties of supercritical fluids near their critical points is widely used in industry [67]. For example, supercritical carbon dioxide, having a critical temperature of 304.2 K and pressure of 73.8 atm, is used to decaffeinate coffee. Supercritical water below the Earth's surface leads to the formation of many of the attractive crystals that are used in jewelry.

Little is known about the behavior of organic molecules in supercritical dihydrogen as a solvent. In the 1950s and 1960s, various laboratories studied the solubility of organic molecules (e.g. naphthalene) in compressed gases, including dihydrogen and helium [68,69]. None of the environments examined in the laboratory explored high pressures and temperatures, however.

Throughout most of the volume of gas giant planets where molecular dihydrogen is stable, it is a supercritical fluid. For most of the volume, however, the temperature is too high for stable carbon–carbon covalent bonding. We may, however, define two radii for each of the gas giants. The first is the radius where dihydrogen becomes supercritical. The second is where the temperature rises to a point where organic molecules are no longer stable; for this discussion, this is chosen to be 500 K. If the second radius is smaller than the first, then the gas giant has a 'habitable zone' for life in supercritical dihydrogen. If the second radius is larger than the first, however, then the planet has no habitable zone.

If such a zone exists on Jupiter, it is narrow. Where the temperature is 300 K (clearly suitable for organic molecules), the pressure (ca. 8 atm) is still subcritical. At about 200 km down, the temperature rises above 500 K, approaching the upper limit where carbon–carbon bonds are stable [70]. For Saturn, Uranus and Neptune, however, the habitable zone appears to be thicker (relative to the planetary radius). On Saturn, the temperature is ca. 300 K when dihydrogen becomes supercritical. On Uranus and Neptune, the temperature when dihydrogen becomes supercritical is only 160 K; organic molecules are stable at this temperature.

The atmospheres of these planets convect. To survive on Jupiter, hypothetical life based on carbon–carbon covalent bonds would need to avoid being moved by convection to positions in the atmosphere where they can no longer survive. This is, of course, true for life in any fluid environment, even in Terran oceans. Sagan and Salpeter presented a detailed discussion of what might be necessary for a 'floater' to remain stable in Jupiter's atmosphere [71].

Life in Terran-like environments

Weird life might exist in weird environments. But we might also ask whether a common chemical model is found in life in Terran-like environments. Here, carbon is the scaffold and water is the solvent. By constraining our discussion to these environments, we fall into line with most literature, which is weighted heavily towards these as being required for life. Thus, in its search for signs of life on Mars, NASA has chosen to follow the water. Given what we know, this seems to be a sensible strategy.

Water as the solvent

Water certainly appears to be required for Terran life, which appears to live only under conditions where water is a liquid. Thus, the extremophiles that live above 373K on the ocean floor do so only because high pressures there keep water liquid. Bacteria in the Antarctic ice pack presumably require melting to grow [72–74].

As a test for the hypothesis that water is required for life, places on Earth that lack water might be searched to see if life has evolved to fill those environments. McKay and his colleagues have searched for life carefully in the Atacama desert, one of the driest spots on Earth [75]. They have concluded that life here is very scarce; assays using PCR failed to detect typical Terran biosignatures, and the number of cultures arising from samples from the driest area is at or near background.

Virtually every environment on Earth that has been examined, from deep in mine shafts to deep in oceans, in environments dominated by reductants as strong as hydrogen sulfide and methane to oxidants as strong as dioxygen, seems to hold life that has evolved from the universal ancestor of all life on Earth. As long as water is available, life finds a way to exploit whatever thermodynamic disequilibria exists [76–78].

This experience suggests that Terran life needs water. But we can still ask whether water is specifically needed for life, or whether the Atacama desert example simply shows that *some* solvent is needed (as no other solvent is present in the Atacama). Once again, we have little experimental information to support an opinion one way or the other. But the properties of water are well known, and we can at least ask whether there is a common chemical model for life in water.

Advantages and disadvantages of water

Most of the literature in this area comments on properties of water that make it well suited for life. We can construct for each a 'good news–bad news' dialectic.

Water, for example, expands when it freezes. This is useful to process rocks to make soils. This expansion also means that ice floats, permitting surface ice to insulate liquid water beneath, which can harbor life on an otherwise freezing planet. Ammonia and methane are both denser as solids than as liquids. They therefore shrink when they freeze, exposing the liquid surface to further freezing.

Another feature of liquid water is the hydrophobic effect. This rubric captures the empirical observation that water and oil do not mix. This is a manifestation of the fact that water forms stronger hydrogen bonds to other water molecules than to oily molecules. The hydrophobic effect is key to the formation of membranes, which in turn support isolation strategies in Terran life. Likewise, when proteins fold, they put their hydrophobic amino acids inside, away from water.

On the other hand, water engages in undesired reactions as well. Thus, cytidine hydrolytically deaminates to give uridine with a half-life of ca. 70 years in water at 300 K [79]. Adenosine hydrolytically deaminates (to inosine), and guanosine hydrolytically deaminates to xanthosine at only slightly slower rates. As a consequence, Terran DNA in water must be continuously repaired. The toxicity of water creates special problems for the prebiotic chemistry, as repair mechanisms presumably require a living system.

Universal constraints for life in water

Water also constrains the structure of carbon-containing biomolecules and their subsequent metabolism. At the very least, for life to exploit the power of water as a solvent, many biomolecules must be soluble in water. This is the simplest explanation for the prevalence of hydroxyl groups in organic molecules central to biological metabolism. Glucose has five, and dissolves well in water.

Charge also helps dissolution in water. Many intermediates in Terran metabolism are phosphorylated, giving them charges that increase solubility in water. The citric acid cycle is based on tri- and dicarboxylic acids. These are ionized at physiological pH, making them soluble in water.

DNA, the molecule at the center of Darwinian evolution on Earth, shows the importance of being soluble [80]. The DNA duplex, where a polyanion binds another polyanion, appears to disregard Coulomb's law. One might think (and indeed, many have thought) [81,82] that the duplex would be more stable if one strand were uncharged, or polycationic [83,84].

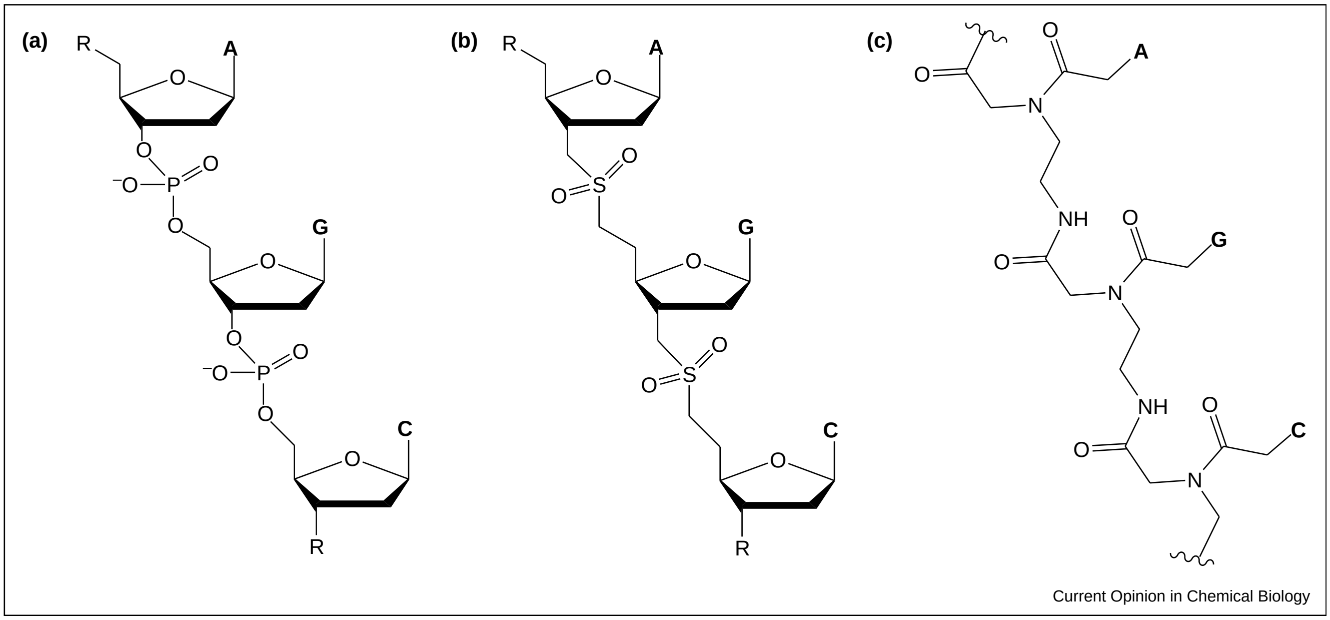
Many efforts have been made to create non-ionic analogs of DNA and RNA [82]. For example, replacing the anionic phosphate diester linker with the uncharged dimethylenesulfone linker generates DNA and RNA analogs that are rough disasters of the phosphate analog (Figure 5) [85–87]. Short sulfone-linked DNA analogs (SNAs) display molecular recognition of the Watson–Crick type [88]. In longer oligosulfones, however, the loss of the repeating charge damages rule-based molecular recognition [86,89]. Further, SNAs differing by only one nucleobase displayed different levels of solubility, aggregation, folding and chemical reactivity [90,91].

These results suggest three hypotheses for why charged phosphate linkages are important to molecular recognition in DNA. First, the repeating charges in the backbone force inter-strand interactions away from the backbone, causing the strands to contact at the Watson–Crick edge of the heterocycles (Figure 6). Without the polyanionic backbone, inter-strand contacts can be anywhere [92]. Further, the repeating charges in the backbone keep DNA strands from folding. A flexible polyanion is more likely to adopt an extended conformation suitable for templating than a flexible neutral polymer, which is more likely to fold [93].

Last, the repeating backbone charges allow DNA to support Darwinian evolution. As noted above, replication is not sufficient for a genetic molecule to support Darwinian evolution. The Darwinian system must also generate inexact replicates, descendants whose chemical structures are different from those of their parents. These differences must then be replicable themselves. While self-replicating systems are well known in chemistry, those that generate inexact copies, with the inexactness itself replicable, are not [94]. Indeed, small changes in molecular structure often lead to large changes in the physical properties of a system. This means that inexact replicates need not retain the general physico-chemical properties of their ancestors, in particular, properties that are essential for replication.

In DNA, the polyanionic backbone dominates the physical properties of DNA. Replacing one nucleobase by another, therefore, has only a second-order impact on the

Figure 5



Replacing **(a)** the anionic phosphate diester linker with **(b)** the uncharged dimethylenesulfone linker generates DNA and RNA analogs that are rough isosteres of the phosphate analog. **(c)** Peptide nucleic acid (PNA) is a DNA/RNA mimic in which the phosphate–sugar backbone has been replaced by uncharged *N*-(2-aminoethyl)glycine linkages with the nucleobases attached through methylene carbonyl linkages to the glycine amino group.

physical behavior of the molecule. This allows nucleobases to be replaced during Darwinian evolution without losing properties essential for replication.

For this reason, a repeating charge may be a universal structure feature of any genetic molecule that supports Darwinian evolution in water [85]. Polycationic backbones may be as satisfactory as polyanionic backbones, however [82,83]. Thus, if life is detected in water on other planets, their genetics are likely to be based on polyanionic or polycationic backbones, even if their nucleobases differ from those found on Earth. This structural feature can be easily detected by simple analytical devices.

A repeating dipole as a universal structural feature of catalysts in water

As noted above, the specific 20 amino acids that are common in standard Terran proteins need not be universal. But what about the amide backbone? Unlike DNA and RNA, where the repeating element is a monopole (a charge), the repeating element of a polypeptide chain is a dipole. This is ideal for folding; the positive ends of one dipole interact favorably with the negative end of another dipole. By comparison, a biopolymer based on a repeating ester linkage would not fold via backbone-backbone interactions (Figure 7). As folding is almost certainly required for efficient catalysis, one might expect repeating dipoles as found in polyamides to be found throughout the galaxy in biospheres that are based on water.

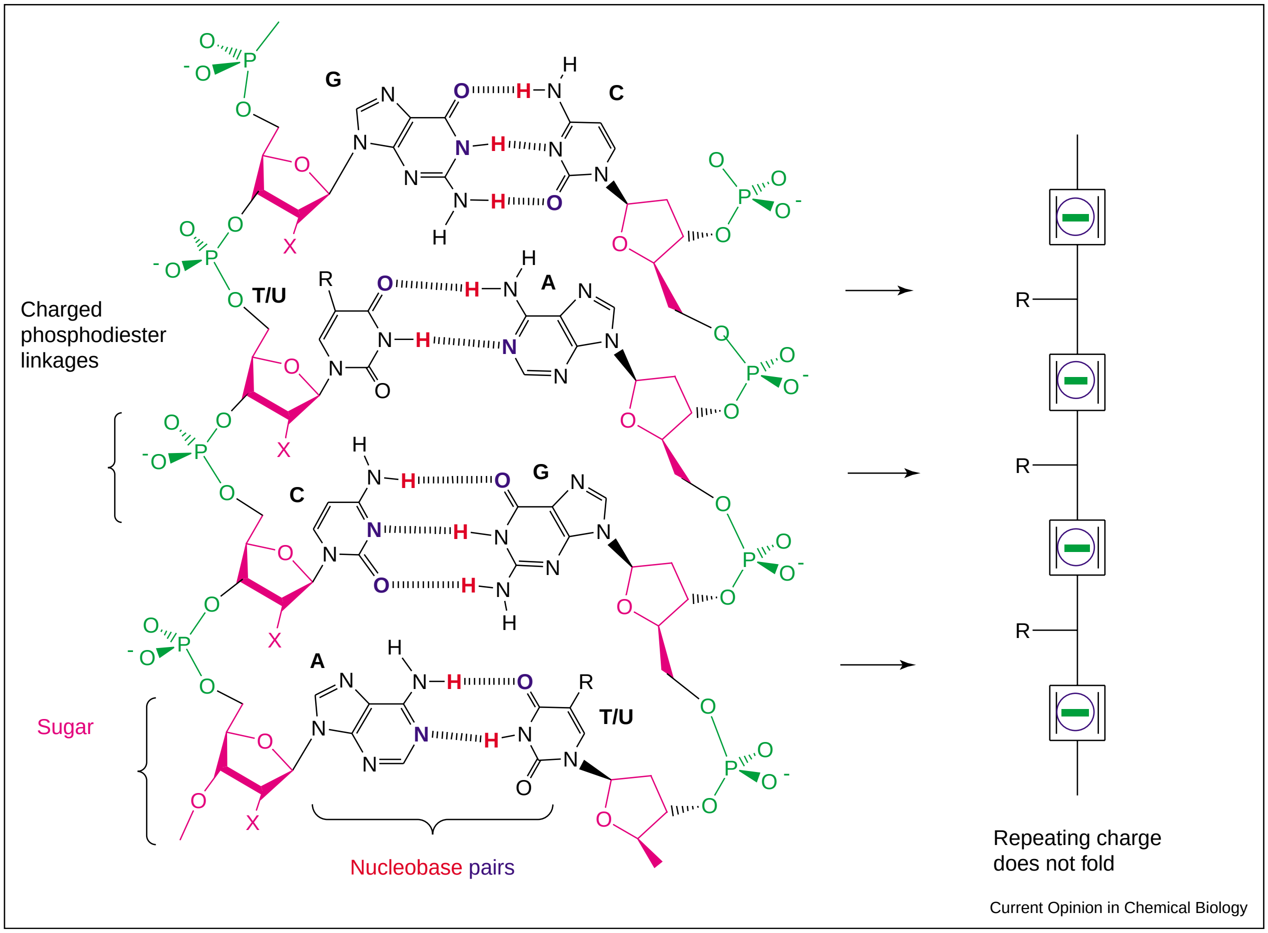
Amino acids appear to be products of prebiotic synthesis. Further, they are found in carbonaceous meteorites, both as α amino acids that are standard in Terran life, and in the non-standard α-methyl amino acids found by Cronin and Pizzarello [95].

The uniqueness of bonding between carbon, oxygen and nitrogen makes it difficult to conceive of an alternative backbone that contains a repeating dipole. Selecting from the third row of the periodic table, repeating sulfonamide certainly would be one possibility (Figure 7) [96]. Repeating phosphonamides, where the negative charge is blocked, would be another alternative [97].

Universal structural features of metabolites in water

The strength of the carbon–carbon bond that makes it valuable to support covalent bonding becomes a disadvantage when it comes to metabolism. Bonds between two carbon atoms do not break at temperatures where water is liquid unless they have reason to do so. In particular, a reactant must be structured so that the electron pair that forms the bond between two carbon atoms has a place to go other than on to the carbon itself. In modern Terran metabolism, the electrons generally move, directly or indirectly, to an oxygen atom that begins

Figure 6



The repeating charges in the backbone force inter-strand interactions away from the backbone, causing the strands to contact at the Watson–Crick edge of the heterocycles. Without the polyanionic backbone, inter-strand contacts can be anywhere.

the reaction doubly bonded to a carbon atom. The C=O (carbonyl) group is central to all of contemporary Terran metabolism.

Other atoms can be doubly bonded to carbon and generate similar reactivity. For example, nitrogen doubly bonded to carbon supports much of the same chemistry. In water, the C=N (imine) unit is rapidly hydrolyzed to give an amine and a carbonyl-containing product containing a C=O at the corresponding position. Therefore, in modern Terran biochemistry, the imine is a transient species in many chemical reactions, including those that occur in an active site of the enzyme. Pyridoxal chemistry is a good example of this.

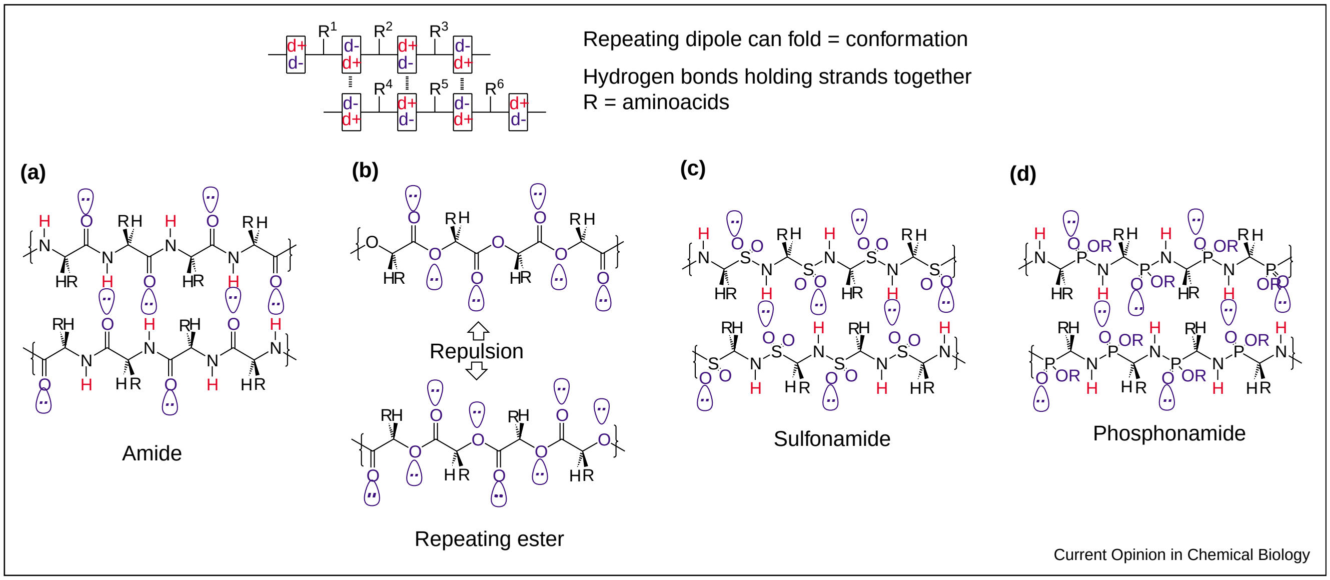
Universal problems in water

Many organic molecules that are nearly inescapably parts of a carbon-based metabolism display problematic reactivity in water. Life that does not escape these molecules must deal with their reactivity.

For example, carbon dioxide is a key to carbon cycling in any moderately oxidizing environment. Water and carbon dioxide are a problematic pair, however. The carbon of carbon dioxide is a good electrophilic center. But carbon dioxide itself is poorly soluble in water [0.88 v/v at 293 K and 1 atm], and dissolves at pH 7 primarily in the form of the bicarbonate anion. This, however, has its electrophilic center shielding by the anionic carboxylate group. This means that bicarbonate is intrinsically unreactive as an electrophilic. Thus, the metabolism of carbon dioxide is caught in a conundrum. The reactive form is insoluble; the soluble form is unreactive.

Terran metabolism has worked hard to manage this conundrum. The reactivity of the biotin cofactor was discussed nearly three decades ago in this context [98]. Biotin is metabolically expensive, however, and cannot be used to manage carbon dioxide and its problematic reactivity in large amounts. The enzyme ribulose bisphosphate carboxylase attempts to manage the problem

Figure 7



A repeating dipole (amide) in polypeptides is ideal for folding in water. **(a)** The polyamide found in natural proteins. **(b)** A polyester linkage by contrast will not fold. If we select elements from the third row of the periodic table, **(c)** repeating sulfonamide certainly would be one possibility and **(d)** a repeating phosphonamide in which the charge of the phosphate has been cancelled (as in a phosphosester) will be another.

without biotin. But here, the problematic reactivity of carbon dioxide competes with the problematic reactivity of dioxygen. Even in highly advanced plants, a sizable fraction of the substrate intended to capture carbon dioxide is destroyed through reaction with dioxygen [99]. Terran life, and nearly a billion years, has not found a compelling solution to this problem, which may be universal. Indeed, if we encounter non-Terran carbon-based life, it will be interesting to see how they have come to manage the unfortunate properties of carbon dioxide.

DNA nucleobases as an illustration of the trialectic

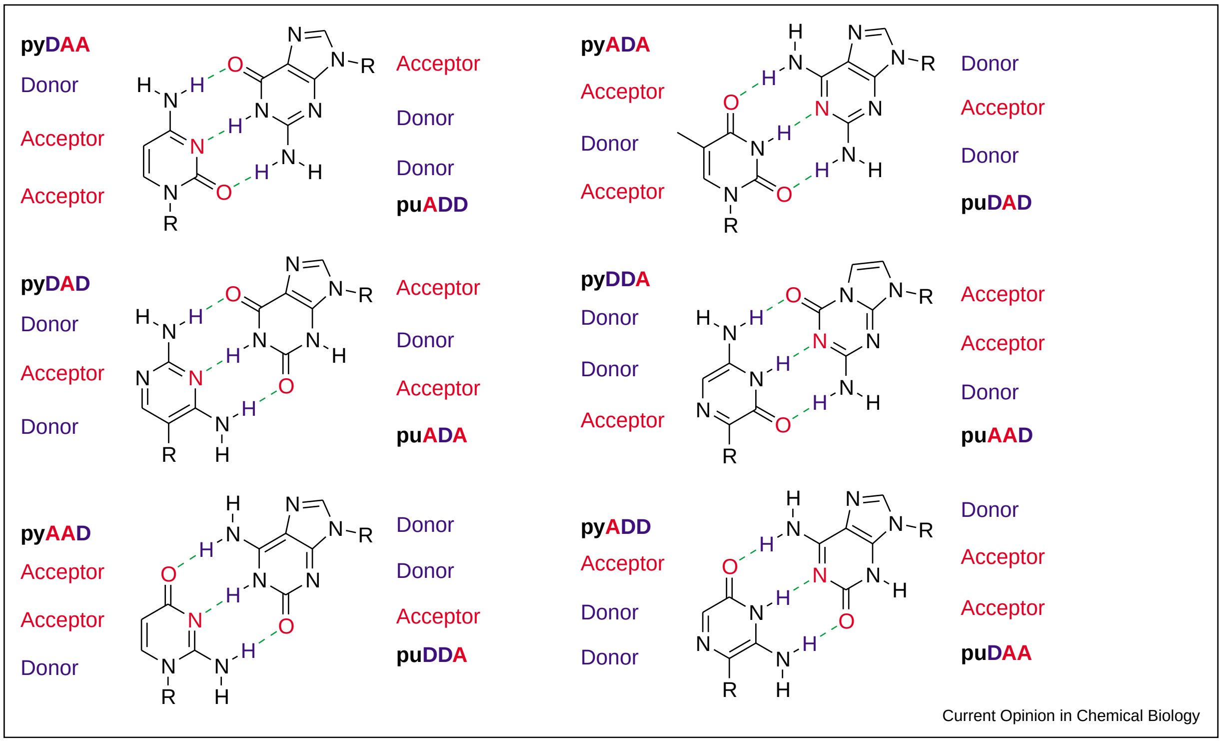
The four standard Terran nucleobases pair following simple rules (A pairs with T, G pairs with C). These, in turn, follow from two rules of complementarity: size complementarity (large purines pair with small pyrimidines), and hydrogen bonding complementarity (hydrogen bond donors pair with hydrogen bond acceptors; Figure 8). This perspective shows that 12 letters forming six mutually exclusive base pairs are possible within the Watson–Crick geometry, obeying size and hydrogen bonding complementarity [100]. Representative examples of each of these have been synthesized, and base pairing is indeed observed following an expanded set of Watson–Crick base pairing rules. The extra letters in an artificial genetic alphabet are useful. They are now used in FDA-approved clinical diagnostics tests that monitor the load of HIV and hepatitis C viruses in ca. 400 000 patients annually [101]. An expanded genetic alphabet also supports the polymerase chain reaction [102], thereby enabling Darwinian evolution with a non-Terran genetic molecule.

Given that these two rules of complementarity have manipulative value in creating artificial genetic systems under standard Terran aqueous conditions, they might also under extreme conditions. Consider, for example, the problem of genetics at low pH. Adenine is half protonated at pH 3.88; cytidine is half-protonated at pH 4.56. Protonation changes the hydrogen bonding patterns of these nucleobases, and disrupts the Watson–Crick pairing that holds the double helix together. Therefore, these nucleobases will not sustain life at low pH, such as in the Venusian atmosphere.

Reflecting this, modern Terran organisms that live at low pH (pHs as low as 0 are known [103]) pump protons to maintain an intracellular pH well above the pK_a of the protonated nucleobases. They could, however, have altered the structure of the nucleobases to alter their basicity. For example, 5-nitrocytidine has a pK_a that should be considerably lower than that of cytidine.

Why have Terran organisms that live at low pH chosen to pump protons rather than alter their DNA nucleobases? A functional explanation would hold that the fitness lost through a requirement to pump protons is less than the fitness lost through the biosynthesis of alternative nucleobases. An explanation based on vestigiality accepts that replacing cytidine by 5-nitrocytidine might indeed

Figure 8



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Expanded genetic alphabet. Following the rules of size complementarity (large purines pair with small pyrimidines) and hydrogen bonding complementarity (hydrogen bond donors from one nucleobase pair with hydrogen bond acceptors from another), the four Terran nucleobases are a minority of the 12 that can form mutually exclusive pairs that fit the Watson–Crick base pairing geometry.

generate an organism that is more fit, but that the life that invaded the low pH environments had cytidine, not 5-nitrocytidine, together with the metabolism to make and degrade it, the polymerases to copy it, and the molecular biology to use it. Changing all of this was not possible using Darwinian processes.

An analogous discussion extends to the ribose in Terran RNA [104]. Evidence for an ‘RNA world’, an episode of life on Earth where RNA was the only genetically encoded component of biological catalysts [105], is found in the ribosome [106], in catalytic RNA molecules [107–109], and in RNA fragments from contemporary metabolism [110]. Many have doubted, however, that the first forms of life used RNA as its genetic material [111]. This doubt arises in part because of the chemical instability of ribose in water [112].

Ribose can be made abiologically. When incubated with technical calcium hydroxide, formaldehyde and glycolaldehyde (both in the interstellar medium [113,114]) are converted into pentoses, including ribose, in a transformation known as the formose reaction [115,116]. Unfortunately, the ribose rapidly suffers degradation under these conditions. Threose, explored as an alternative to ribose [117], does also.

To address the stability problem, ribose phosphates have been proposed [118]. Further, borates stabilize ribose under conditions where it is formed [61]. Boron is not a common element on Earth. Yet, borate is excluded from many silicate minerals and enriched in igneous melts. Further, boron is enriched in the crust over its concentration in the mantle. Thus, sediments can contain as much as 100–150 ppm boron [119], and its appearance in subduction volcanoes is taken as evidence of their origin. Boron also appears in igneous rocks on the surface as tourmalines. Alkaline borate minerals are well known on Earth. Serpentinization of olivine in mantle rocks generates alkaline solutions [120]. Weathering processes deliver borate to aqueous solution, the oceans, and to evaporite minerals [121].

The prebiotic synthesis of sugars is now being examined with renewed interest. Non-racemic amino acid mixtures and zinc-proline complexes act as asymmetric catalyst during the condensation of glycolaldehyde and formaldehyde [122,123]. Ribose has been shown to be selectively precipitated by derivatization reaction with cyanamide. When the racemic mixtures of D-, and L-ribose were reacted with cyanamide, pure crystals containing homochiral domains were observed [124]. These recent discoveries make plausible the vestigial role of ribose in the origins of life.

Non-Terran life on Earth

Does an alternative biosphere exist on Earth? This question is not as absurd as it might seem. Just 50 years ago, the Archaeal kingdom was not recognized. Life deep in the ocean was not known. With over 90% of microorganisms not culturable, we cannot exclude the possibility that Earth still carries a form of life that we do not already know of. Indeed, as this manuscript was being written, a new macroscopic, multicellular worm was discovered feeding on whale carcasses two miles below the Pacific Ocean [125].

This question can be approached from two extremes. First, we can ask whether a life form that many believe was certainly possible still remains on Earth. The strongest current view for the natural history of life suggests that it began as part of an RNA world, a chemical system capable of Darwinian evolution that used RNA as the sole genetically encoded component of biological catalysis [105,126]. We might ask: do RNA organisms still exist on Earth?

It is not clear that we would have found them if they were here. First, virtually all of the 'universal' probes for life target the ribosome, a molecule that makes encoded proteins. The ribosome is, almost by definition, not present in RNA organisms.

Where on Earth might environments be found where RNA organisms have a selective advantage, and therefore may have survived the 2 billion years of competition with protein based life? Three thoughts come to mind. First, the encoded biopolymer of RNA organisms does not require sulfur; proteins do. RNA organisms may therefore survive in an environment depleted in sulfur.

Alternatively, ca. 70% of the volume of a typical protein-based three polymer microorganism is consumed by the translation machinery needed to make proteins. This machinery is not needed by an RNA organism. Therefore, RNA organisms can be much smaller than protein-based organisms [127]. This suggests we might look for RNA organisms on Earth by looking for environments that are space-constrained. Many minerals have pores that are smaller than one micron across. These might hold smaller RNA organisms.

RNA is also easier to denature and then refold, compared with proteins. This might create a niche for an RNA organism in environments that cycle between very high and very low temperatures.

Conclusion

This review suggests that life might exist in a wide range of environments. These include non-aqueous solvent systems at low temperatures (such as found on Saturn's moon Titan). Life may even exist in more exotic environments, such as the supercritical dihydrogen–helium mixtures found on gas giants. We propose that the only absolute requirements are a thermodynamic disequilibrium and temperatures consistent with chemical bonding. Weaker desiderata include a solvent system, the availability of elements such as carbon, hydrogen, oxygen and nitrogen, certain thermodynamic features available to metabolic pathways, and the opportunity for isolation. If we constrain life to water, more specific criteria can be proposed, including soluble metabolites, genetic materials with repeating charges, and a well defined temperature range.

Acknowledgements

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