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Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism

Bhavesh H. Patel, Claudia Percivalle, Dougal J. Ritson, Colm D. Duffy & John D. Sutherland

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Abstract

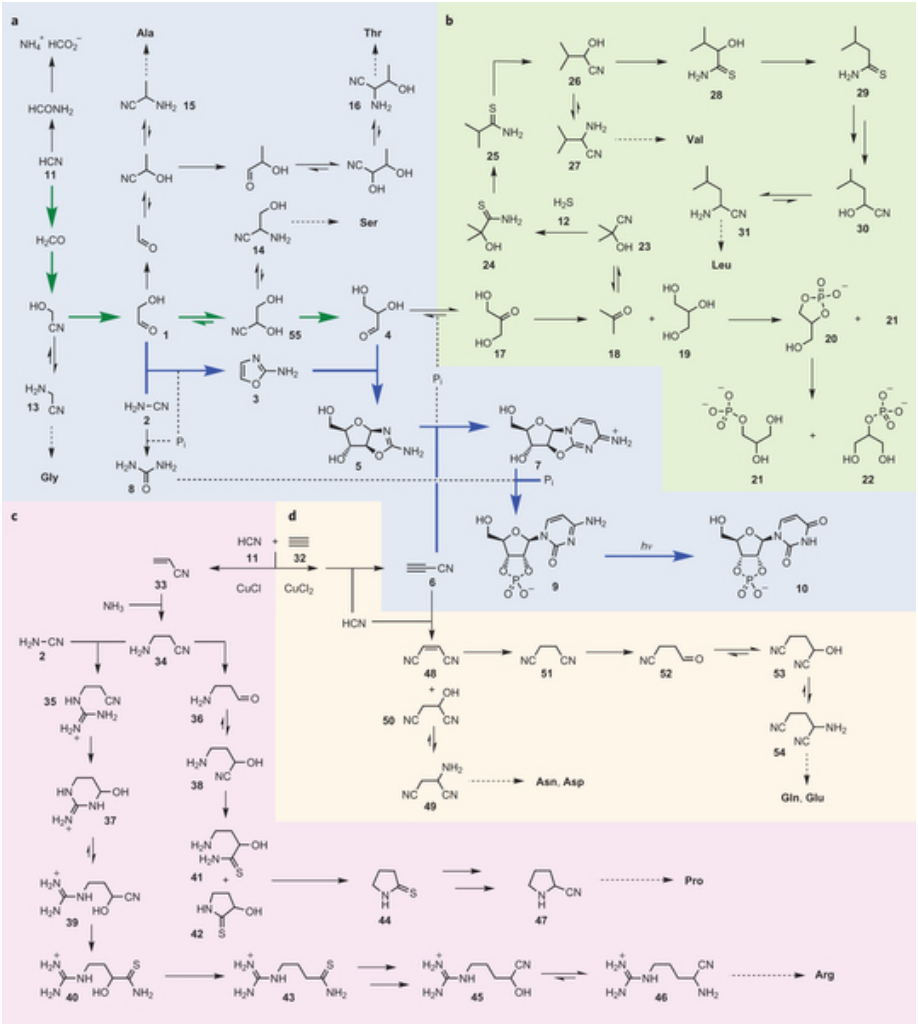
Print

A minimal cell can be thought of as comprising informational, compartment-forming and metabolic subsystems. To imagine the abiotic assembly of such an overall system, however, places great demands on hypothetical prebiotic chemistry. The perceived differences and incompatibilities between these subsystems have led to the widely held assumption that one or other subsystem must have preceded the others. Here we experimentally investigate the validity of this assumption by examining the assembly of various biomolecular building blocks from prebiotically plausible intermediates and one-carbon feedstock molecules. We show that precursors of ribonucleotides, amino acids and lipids can all be derived by the reductive homologation of hydrogen cyanide and some of its derivatives, and thus that all the cellular subsystems could have arisen simultaneously through common chemistry. The key reaction steps are driven by ultraviolet light, use hydrogen sulfide as the reductant and can be accelerated by Cu(I)–Cu(II) photoredox cycling.

Main

Viewing the cell as an ensemble of subsystems[1] begs the question 'did the subsystems emerge together, or one after the other at the origin of life?'. The consensus that sequential emergence is more probable[2] (although with opinions differing as to which subsystem came first[3, 4, 5]) is based on the notion that different, mutually incompatible, chemistries are needed to make the various subsystems. We set out to explore this experimentally by evaluating the assembly chemistry of the various subsystems[6, 7]. Investigation of the assembly chemistry of an informational subsystem based on RNA led to our discovery of an efficient synthesis of activated pyrimidine ribonucleotides[6]. In this synthesis (Fig. 1a, bold blue arrows), the C_2 sugar glycolaldehyde (**1**) undergoes phosphate-catalysed condensation with cyanamide (**2**) to give 2-aminooxazole (**3**). This heterocycle then participates in a C–C bond-forming reaction with the C_3 sugar glyceraldehyde (**4**), which gives rise to a mixture of pentose aminooxazolines. Reaction of the *arabino*-configured aminooxazoline (**5**) with cyanoacetylene (**6**) then furnishes the anhydronucleoside **7**, which on heating with phosphate in urea (**8**), a by-product of the first step of the sequence, is transformed into *ribo*-cytidine-2′,3′-cyclic phosphate (**9**). Ultraviolet irradiation then partially converts this nucleotide into uridine-2′,3′-cyclic phosphate (**10**) and destroys stereoisomeric impurities.

Figure 1: Reaction network that leads to RNA, protein and lipid precursors.



The degree to which the syntheses of ribonucleotides, amino acids and lipid precursors are interconnected is apparent in this 'big picture'. The network does not produce a plethora of other compounds, however, which suggests that biology did not select all of its building blocks, but was simply presented with a specific set as a consequence of the (photo)chemistry of hydrogen cyanide (**11**) and hydrogen sulfide (**12**), and that set turned out to work. To facilitate the description of the chemistry in the text, the picture is divided into four parts. **a**, Reductive homologation of hydrogen cyanide (**11**) (bold green arrows) provides the C_2 and C_3 sugars—glycolaldehyde (**1**) and glyceraldehyde (**4**)—needed for subsequent ribonucleotide assembly (bold blue arrows), but also leads to precursors of Gly, Ala, Ser and Thr. **b**, Reduction of dihydroxyacetone (**17**) (the more stable isomer of glyceraldehyde (**4**)) gives two major products, acetone (**18**) and glycerol (**19**). Reductive homologation of acetone (**18**) leads to precursors of Val and Leu, whereas phosphorylation of glycerol (**19**) leads to the lipid precursor glycerol-1-phosphate (**21**). **c**, Copper(I)-catalysed cross-coupling of hydrogen cyanide (**11**) and acetylene (**32**) gives acrylonitrile (**33**), reductive homologation of which gives precursors of Pro and Arg. **d**, Copper(II)-driven oxidative cross-coupling of hydrogen cyanide (**11**) and acetylene (**32**) gives cyanoacetylene (**6**), which serves as a precursor to Asn, Asp, Gln and Glu. P_i, inorganic phosphate.

We subsequently showed that the C_2 and C_3 sugars **1** and **4** can be provided sequentially by a Kiliani–Fischer-type homologation of hydrogen cyanide (**11**) using Cu(I)–Cu(II) photoredox chemistry (Fig. 1a, bold green arrows)[8, 9]. Using hydrogen sulfide (**12**) as the stoichiometric reductant—in which case the inclusion of Cu(I) is no longer essential—we further found that **13**–**16**, the α-aminonitrile Strecker precursors of amino acids glycine, serine, alanine and threonine, are inevitable by-products of this RNA assembly chemistry[9], and thereby strengthen its apparent aetiological relevance. However, we felt that the discovery of routes to other biologically relevant compounds would make the case even stronger and, accordingly, we further explored this area of chemistry.

Results and discussion

Triose-derived building blocks

The involvement of glyceraldehyde (**4**) and phosphate in the scheme prompted us to consider the interconversion of **4** and its more stable triose isomer, dihydroxyacetone (**17**), and to investigate the chemistry of the latter (Fig. 1b). The interconversion of **4** and **17** can occur by enolization–ketonization[10], and we reasoned that it might be subject to general acid–base catalysis by phosphate. Accordingly, we incubated glyceraldehyde (**4**) in a near-neutral pH phosphate buffer and found that it slowly but smoothly converted into dihydroxyacetone (**17**) (Table 1). We then subjected **17** to photoreduction by hydrogen sulfide (**12**) and observed two major products, acetone (**18**) and glycerol (**19**). The biological relevance of glycerol (**19**) as a lipid precursor is obvious, but we could also see in the geminal methyl groups of acetone (**18**) a possible link with natural products containing an isopropyl moiety. Focusing first on glycerol (**19**), we subjected it to the same conditions that we had previously used for the conversion of anhydronucleoside **7** into nucleotide **9**, and found that it is efficiently converted into a mixture that contains glycerol-1,2-cyclic phosphate (**20**) and glycerol-1-phosphate (**21**). The cyclic phosphate is strained and therefore prone to hydrolytic ring-opening; however, uncatalysed hydrolysis is slow. Divalent transition metal ions are known to catalyse phosphotransfer reactions[11] and so we treated the glycerol phosphorylation products with Zn(II) after which **21** and the isomeric glycerol-2-phosphate (**22**) were obtained in good yield (Table 1). The major membrane-forming amphiphiles of all three kingdoms of life are esters or ethers of glycerol-1-phosphate (**21**)[12], and the finding that **21** can be efficiently synthesized from the RNA intermediate, glyceraldehyde (**4**), suggests that the link between the informational and compartment-forming subsystems might start with the synthesis of their building blocks.

Table 1: Yields for the part of the reaction network shown in Fig. 1b**.**

Returning now to acetone (**18**), the other major product of the reduction of dihydroxyacetone (**17**), we wondered if it might undergo the Kiliani–Fischer-type homologation chemistry. However, the equilibrium for the formation of cyanohydrin **23** from ketone **18** and hydrogen cyanide (**11**) is not as favourable as it is in the case of an aldehyde[13], and when we subjected the equilibrium mixture to the photoreduction using hydrogen sulfide (**12**), we found that hydrogen cyanide (**11**) and acetone (**18**) are reduced instead of cyanohydrin **23**. Reasoning that the introduction of hydrogen sulfide (**12**) into the system need not necessarily be at the same time as the irradiation, we next investigated the addition of **12** to the ketone–cyanohydrin equilibrium mixture prior to irradiation. It transpires that cyanohydrin **23** is more reactive than hydrogen cyanide (**11**) towards attack by hydrosulfide (HS^-, the conjugate base of hydrogen sulfide (**12**)) at neutral pH in this 'dark' reaction, and α-hydroxythioamide **24** is formed. Furthermore, as cyanohydrin **23** is consumed, the equilibrium that produces it from acetone (**18**) and **11** is displaced according to Le Chatelier's principle, with the effect that more **24** is produced than there is cyanohydrin **23** at equilibrium. Irradiating the reaction products for a limited period of time causes clean deoxygenation of α-hydroxythioamide **24** to give thioamide **25**. This latter thioamide is reduced to the corresponding aldehyde by continued irradiation in the presence of hydrogen sulfide (**12**), but further reduction of the aldehyde proved to be competitive, and so we carried out the reduction in the presence of hydrogen cyanide (**11**), whereupon the aldehyde was trapped as its cyanohydrin (**26**). Clearly, **26** is constitutionally related to **27**, the α-aminonitrile precursor of valine, as we demonstrated through conversion of the former into the latter by the addition of ammonia, but we could now see that a further cycle of homologation might furnish the corresponding precursor of leucine too. Thus, dark reaction with hydrogen sulfide (**12**) converts cyanohydrin **26** into α-hydroxythioamide **28**, and subsequent irradiation of the reaction products causes the deoxygenation of **28** to give thioamide **29**. Further reduction in the presence of **12** and hydrogen cyanide (**11**) gives cyanohydrin **30** that, on the addition of ammonia, furnishes the leucine α-aminonitrile precursor **31**.

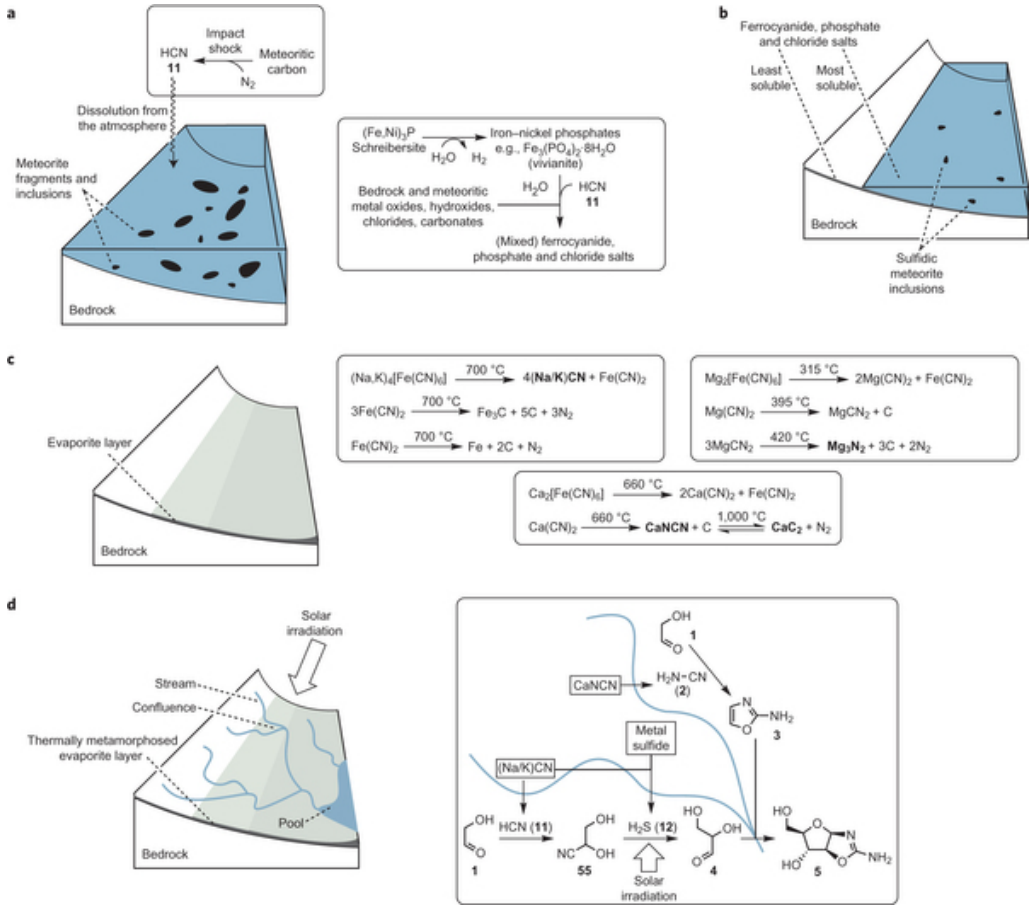
Towards a geochemical scenario

The finding that so many biologically relevant compounds can stem from hydrogen cyanide (**11**) now forced us to consider a geochemical source for **11**. The very specific requirements of the reaction network—the additional need for cyanamide (**2**), cyanoacetylene (**6**), phosphate and hydrogen sulfide (**12**) under conditions including ultraviolet irradiation in aqueous solution—considerably narrowed our search for an outline scenario, and we hoped to be rewarded with (thus far) missing reagents, feedstocks for the synthesis of other biomolecules and clues as to how to overcome the requirement for a sequential reagent delivery.

Evidence suggests that life started during, or shortly after the abatement of, the Late Heavy Bombardment, and processes associated with meteorite impact have been implicated in the generation of hydrogen cyanide (**11**) and phosphate on the Hadean Earth. Thus, **11** is produced by impact through a high-temperature reaction of carbonaceous meteoritic material with atmospheric nitrogen[14], and the anoxic

corrosion of schreibersite ($(Fe,Ni)_3P$), a mineral that tends to rim metal sulfide inclusions in iron–nickel meteorites), in surface water has been suggested as a source of phosphate, albeit as insoluble transition-metal salts[15, 16]. It has been suggested separately that atmospheric hydrogen cyanide (**11**) could be captured by a gradual dissolution in surface water and coordination to ferrous ions to give ferrocyanide[17], although the recovery of free cyanide by photoaquation, as proposed, is unlikely to have generated concentrated solutions of **11** because of a rapid back reaction[18]. Despite this latter problem, we were attracted to this mode of capture of hydrogen cyanide (**11**) because it could be coupled to the solubilization of phosphate if vivianite (the corrosion product of the insoluble $Fe_{(II)}$ phosphate schreibersite[19]) was one of the sources of ferrous ions (Fig. 2a). Accordingly, we wondered if there were other ways in which cyanide could be recovered from ferrocyanide, and found literature reports that heating the sodium or potassium salts of ferrocyanide to high temperatures generates sodium or potassium cyanide, (Na/K)CN, along with iron carbide and carbon[20, 21]. In our outline geochemical scenario, this would correspond to the evaporation of a body of water that contained ferrocyanides, among other salts, and result in the deposition of an evaporite layer comprising the solid salts, followed by thermal metamorphosis as a consequence of geothermal activity or impact heating (Fig. 2b,c). Interestingly, the group II ferrocyanide salts give different thermal decomposition products in addition to iron carbide and carbon[20, 22]: magnesium ferrocyanide gives magnesium nitride (Mg_3N_2) and calcium ferrocyanide gives calcium cyanamide (CaNCN). Furthermore, calcium cyanamide, on heating to ~1,000 °C with carbon, equilibrates with calcium carbide (CaC_2) and nitrogen[23]. This hinted at a way to obtain all the organic feedstocks needed for our developing reaction network by the addition of a limited amount of water to a thermally metamorphosed evaporite layer that initially contained group I and II ferrocyanide salts. Thus, hydration of sodium and potassium cyanide gives the cyanide needed for the homologation chemistry, hydration of calcium cyanamide gives the cyanamide (**2**) needed for the synthesis of 2-aminooxazole (**3**) and hydration of calcium carbide gives acetylene (**32**), which, if it could be oxidatively coupled with hydrogen cyanide (**11**), would give cyanoacetylene (**6**). Hydration of magnesium nitride gives ammonia, which is required alongside **11** for the Strecker synthesis of α-aminonitriles from aldehydes[24], and the reaction of sodium or potassium cyanide solution with certain metal sulfides is known to generate hydrosulfide, the stoichiometric reductant in much of our photoredox chemistry[25, 26]. In addition to iron sulfide, which, like schreibersite, is a meteoritic component[19], copper sulfide could plausibly have been enriched on the surface of the Hadean Earth by impact-triggered hydrothermal processes[27]. The reaction of copper sulfide with a cyanide solution gives cyanocuprates in addition to hydrosulfide[26], and the photoreduction chemistry we have discovered is most efficient with $Cu_{(I)}$–$Cu_{(II)}$ photoredox cycling when using hydrosulfide as the stoichiometric reductant[9].

Figure 2: Chemistry in a post-meteoritic-impact scenario.



A series of post-impact environmental events are shown along with the chemistry (boxed) proposed to occur as a consequence of these events. **a**, Dissolution of atmospherically produced hydrogen cyanide results in the conversion of vivianite (the anoxic corrosion product of the meteoritic inclusion schreibersite) into mixed ferrocyanide salts and phosphate salts, with counter cations being provided through neutralization and ion-exchange reactions with bedrock and other meteoritic oxides and salts. **b**, Partial evaporation results in the deposition of the least-soluble salts over a wide area, and further evaporation deposits the most-soluble salts in smaller, lower-lying areas. **c**, After complete evaporation, impact or geothermal heating results in thermal metamorphosis of the evaporite layer, and the generation of feedstock precursor salts (in bold). **d**, Rainfall on higher ground (left) leads to rivulets or streams that flow downhill, sequentially leaching feedstocks from the thermally metamorphosed evaporite layer. Solar irradiation drives photoredox chemistry in the streams. Convergent synthesis can result when streams with different reaction histories merge (right), as illustrated here for the potential synthesis of arabinose aminooxazoline (**5**) at the confluence of two streams that contained glycolaldehyde (**1**), and leached different feedstocks before merging.

Further chemistry suggested by the geochemical scenario

Considering evaporites and cyanocuprates in the context of the foregoing, we were drawn to the literature concerning the cross-coupling of hydrogen cyanide (**11**) and acetylene (**32**) to give acrylonitrile (**33**)[28] using copper(I) salts solubilized in water by high concentrations of sodium or potassium chloride, a system known as the Nieuwland catalyst. This combination of reagents and salts appeared prebiotically plausible according to our developing geochemical scenario, and we thus concluded that copper-catalysed cross-couplings could have occurred on the early Earth. We were immediately interested by the possibility of effecting the oxidative cross-coupling of **11** and **32** with copper(II) to give cyanoacetylene (**6**), but first explored the chemistry of acrylonitrile (**33**) and other reagents suggested by the scenario (Fig. 1c and Table 2).

Table 2: Yields for the parts of the reaction network shown in Fig. 1c,d**.**

Acrylonitrile-derived building blocks

The addition of ammonia to **33** generates β-aminopropionitrile (**34**)[29], and we realized that this is a potential precursor of proline and lysine if the amino group of **34** was left free, and arginine if the amino group of **34** could somehow be guanidinylated. In an attempt to implement this guanidinylation, we treated β-aminopropionitrile (**34**) with cyanamide (**2**) and observed that it is converted into the guaninylated derivative **35**, but the reaction is relatively inefficient with the result that **35** is generated in an admixture with residual **34** and cyanamide (**2**). Photoreduction of β-aminopropionitrile (**34**) by hydrogen sulfide (**12**) smoothly furnishes β-aminopropionaldehyde (**36**), and we thus expected the corresponding reduction of the mixture of **34** and **35** to give a mixture of **36** and its guanidinylated analogue. When we subjected the mixture to immediate photoreduction, however, we observed only the guanidinylated analogue (in its hemiaminal form (**37**)) and no **36**. It appears that the reduction of **34** in the mixture does occur, but that residual cyanamide (**2**) then reacts rapidly with **36** to give **37**. If, however, there was a delay before the onset of photoreduction, the amount of **2** would drop through dimerization and hydrolysis, and **37** would be formed along with **36** from the mixture of **34** and **35**. Mechanistically, the extraordinarily efficient reaction of β-aminopropionaldehyde (**36**) and cyanamide (**2**) to give **37** is thought to proceed via the rapid, reversible addition of **2** to the carbonyl group of **36** followed by intramolecular guanidinylation. We next subjected aldehyde **36** and hemiaminal **37** to our Kiliani–Fischer-type homologation chemistry, and used the variant in which reduction by hydrogen sulfide (**12**) follows a dark reaction of the cyanohydrin with **12**, simply because it is the most efficient. In the first step of the homologation, the addition of hydrogen cyanide (**11**) gives cyanohydrins **38** and **39** from **36** and **37**, respectively. The addition of hydrogen sulfide (**12**) to cyanohydrin **39** then proceeds as expected to give the α-hydroxythioamide **40**, but the reaction of cyanohydrin **38** proceeds with a twist in that the expected open-chain α-hydroxythioamide **41** is formed alongside the cyclic α-hydroxythioamide **42**. Furthermore, although the subsequent irradiation of the α-hydroxythioamide **40** and hydrogen sulfide (**12**) simply causes deoxygenation to give the thioamide **43**, the corresponding treatment of a mixture of **41**, **42** and **12** also results in further cyclization such that γ-butyrothiolactam (**44**) is the only deoxygenated thioamide observed. Further photoreduction of thioamide **43** followed by the addition of hydrogen cyanide (**11**) then gives cyanohydrin **45** from which **46** (the α-aminonitrile precursor of arginine) is produced on the addition of ammonia. In the case of the cyclic thioamide **44**, further reduction and the addition of **11** directly generates **47**, the α-aminonitrile precursor of proline. In the context of the origin of the proteinogenic amino acids, two features of the chemistry that leads from acrylonitrile (**33**) are of particularly interest. First, cyclization events during the homologation of β-aminopropionaldehyde (**36**) make a further chain extension to the acyclic Strecker precursor of lysine appear unlikely. Second, the especially efficient reaction of β-aminopropionaldehyde (**36**) with cyanamide (**2**) in the reduction of mixtures of nitriles **34** and **35** suggests that **46**, the α-aminonitrile precursor of arginine, would have been produced alongside **47**, the corresponding precursor of proline, if cyanamide (**2**) was present along with ammonia when acrylonitrile (**33**) was generated.

Cyanoacetylene-derived building blocks

We then returned our attention to the possibility of effecting the oxidative cross-coupling of hydrogen cyanide (**11**) and acetylene (**32**) to give cyanoacetylene (**6**) (Fig. 1d). Although the global redox state of the Hadean Earth would normally limit copper to its 0 and I oxidation levels, copper(I) can easily be photooxidized to give copper(II)[30], which could thus have existed, albeit transiently, in sunlit surface locations. As copper(II) is known to bring about the oxidative coupling of **11** to cyanogen and of acetylenes to diacetylenes[31], we wondered if the addition of copper(II) to a Nieuwland catalyst might enable the oxidative cross-coupling of **11** and acetylene (**32**) to give cyanoacetylene (**6**). However, after the addition of copper(II) chloride, hydrogen cyanide (**11**) and acetylene (**32**) to a Nieuwland catalyst, we could not detect any free cyanoacetylene (**6**). The highly concentrated state of these catalysts means that precipitates are often present, however, and we speculated that cyanoacetylene (**6**) might have been produced in the form of its known solid-state copper-coordination compound CuC_3N[32]. If this were the case, it was thought that the addition of further hydrogen cyanide (**11**) would lead to liberation of free **6** through the binding of cyanide ions to copper(I) outdoing the binding of cyanoacetylide anions. Gratifyingly, when we added additional limited amounts of **11** to the reaction mixture, free cyanoacetylene (**6**) could be detected. By differentiating between the hydrogen cyanide (**11**) added at the beginning of the reaction as a reagent from that added at the end to liberate cyanoacetylene (**6**), through the use of a ^{13}C-label, we were able to show that the oxidative cross-coupling of **11** and acetylene (**32**) gives **6** in >25% yield. Recognizing that the liberation of cyanoacetylene (**6**) from its copper complex need not occur through the addition of limited amounts of hydrogen cyanide (**11**), we next considered the consequences of the liberation of **6** by an excess of **11**. Cyanoacetylene (**6**) is known to undergo the addition of **11** and ammonia at alkaline pH values to give maleonitrile (**48**) and **49**, the α-aminonitrile precursor of asparagine and aspartic acid[33]. We simulated the effect of releasing cyanoacetylene (**6**) from CuC_3N using an excess of hydrogen cyanide (**11**) and ammonia at a slightly alkaline pH simply by adding **6** to a solution of these reagents,

whereupon we observed **48**, **49** and cyanohydrin **50** (Fig. 1d and Table 2). At neutral pH, only maleonitrile (**48**) and cyanohydrin **50** are produced. Photoreduction of maleonitrile (**48**), alongside its photoisomer, fumaronitrile, by hydrogen sulfide (**12**) saturates the double bond to give succinonitrile (**51**). Further irradiation in the presence of **12** selectively reduces one nitrile group of succinonitrile (**51**) to give the semialdehyde **52**, presumably because the electron-withdrawing effect of the second nitrile of **51** makes the first nitrile group more reactive than the nitrile group of **52**. Finally, the addition of hydrogen cyanide (**11**) to the semialdehyde **52** gives cyanohydrin **53** from which **54**, the α-aminonitrile precursor of glutamine and glutamic acid, is produced on the addition of ammonia. Thus, by considering a geochemical scenario consistent with the synthesis of the ribonucleotides **9** and **10**, lipid precursor **21** and Strecker α-aminonitrile precursors of six proteinogenic amino acids, we established a firm link to the synthesis of acrylonitrile (**33**) from which α-aminonitrile precursors of two other amino acids can be obtained. Furthermore, the synthesis of **33** led us to discover a highly related synthesis of cyanoacetylene (**6**) that is needed for the synthesis of ribonucleotides **9** and **10**, and that additionally provides α-aminonitrile precursors of four other amino acids. That consideration of the geochemical scenario we have outlined can lead to the discovery of routes to **6** and six additional proteinogenic amino acids strengthens the validity of both the scenario and the reaction scheme.

Comparison with other 'prebiotic' syntheses

At this point, it is worth comparing our approach to uncovering prebiotically plausible syntheses of multiple biologically relevant compounds with previously reported, 'one-pot' syntheses based on presumed geochemical scenarios. Three such syntheses have dominated the experimental chemical investigation of the origin of life: the Miller–Urey experiment[34] (amino acids, or their Strecker precursors, from lightning in a reducing atmosphere), Butlerow's formose reaction[35] (sugars from atmospherically produced formaldehyde raining onto basic minerals) and Oró's synthesis of purine nucleobases[36] (adenine and other heterocycles from polymerization of ammonium cyanide in solution). Although these syntheses proceed in one pot, they are multistep and suffer from low overall yields of biologically relevant products because of unfavoured reactions and/or reaction sequences. Competing reactions also result in numerous non-biological by-products, which means that any subsequent bimolecular reaction chemistry is prone to generate myriad non-biological products and to be plagued by slow kinetics. Furthermore, to progress towards nucleotides, and mixtures of nucleotides and amino acids, some sort of combination of the syntheses is required. However, trying to meld the various scenarios together has been very problematic because the chemistries are so different, and this is one of the reasons that many in the field have assumed that one such synthesis and associated subsystem came first. It was through analysis of these problems that we adopted the approach of attempting to delineate favoured reaction pathways that lead to multiple biologically relevant compounds, and the reaction network that we present herein (Fig. 1) is the result of this strategy. However, we had also originally hoped to be able to find conditions under which the whole network could operate in one pot (our thinking being influenced by the previous syntheses), but our results now suggest that this would be difficult. Although the yields of the individual steps of the network are uniformly good to excellent (Tables 1 and 2), and several multistep reaction sequences still proceed in good yield in one pot, the key Kiliani–Fischer-type homologation chemistry requires the periodic delivery of hydrogen cyanide (**11**) and hydrogen sulfide (**12**), and there are several points in the network at which the sequential delivery of other reagents is required. We therefore extended our thinking beyond traditional 'one-pot' chemistry and considered other chemical synthesis formats, bearing in mind the need for compatibility with our outline geochemical scenario.

Refinement of the geochemical scenario

One way in which **11** and **12** could be delivered periodically involves flow chemistry[37], and we quickly realized that this would be facile in a geochemical setting. Thus, if the terrain onto which the evaporites were deposited and thermally metamorphosed was not flat, then subsequent rainfall would result in rivulets or streams flowing downhill to form pools at depressions in the evaporite basin (Fig. 2d, left). Water flowing over the products of the thermal metamorphosis of sodium or potassium ferrocyanide would leach out highly soluble sodium or potassium cyanide, and result in a concentrated cyanide solution, which would then dissolve any metal sulfides the stream encountered and liberate hydrosulfide. Solar ultraviolet irradiation could then drive a first phase of the reduction chemistry, which would pause when hydrogen cyanide and hydrosulfide in the stream became depleted. Further passage of the solution over ground that contained soluble cyanide salts and metal sulfides could then initiate subsequent phases of the reduction chemistry to result in homologation of the aldehydes produced in the first phase. Additional reagents, such as phosphate, could also be delivered at other points of the reaction network through the dissolution of evaporite salts. A geochemically plausible refinement of the scenario suggests how convergent synthesis could take place if streams with different flow chemistry histories merged (Fig. 2d, right). Thus, if a stream in which the reductive homologation chemistry had

paused at the stage of glycolaldehyde (**1**) (Fig. 1a) and passed over the thermally metamorphosed products of calcium ferrocyanide, leaching out of cyanamide (**2**) would lead to the synthesis of 2-aminooxazole (**3**). Glycolaldehyde (**1**) in a similar stream that, instead, passed over further ground containing cyanide and metal sulfides would be homologated to give glyceraldehyde (**4**) by way of cyanohydrin **55**. If the two streams subsequently merged, reaction of **3** and **4** at the confluence would generate the pentose aminooxazolines, including **5**. If a stream in which glyceraldehyde (**4**) had been synthesized did not merge with a stream containing 2-aminooxazole (**3**), but instead continued passing over ground containing phosphate, cyanide and metal sulfides, the chemistry that leads to glycerol-1-phosphate (**21**) and to **27** and **31**, the α-aminonitrile precursors of valine and leucine (Fig. 1b), would ensue.

It is not possible to predict precisely where various ferrocyanides and other salts would lie in an evaporite basin, although the topography of the basin floor and the solubilities of salts would have played major determining roles. Thus, the most-soluble salts, such as sodium and potassium chloride, and mixed salts would have precipitated from the solution last, and thus been deposited in relatively small areas as the last pools in the depressions on the basin floor dried out. Less-soluble salts and mixed salts would, presumably, have been deposited from larger bodies of water and thus been spread over larger areas (Fig. 2b). When streams first reached the depressions on the basin floor that contained large amounts of sodium and potassium chloride, brine pools would have formed. If the depressions, or the streams that first reached them, also contained copper ions and cyanide, then the formation of Nieuwland catalysts can easily be envisaged. Leaching of the products of high-temperature thermal metamorphosis of calcium ferrocyanide could then have supplied acetylene (**32**) for cross-coupling with hydrogen cyanide (**11**). Copper(I) ions would have catalysed the synthesis of acrylonitrile (**33**) and thence **46** and **47**, the α-aminonitrile precursors of arginine and proline (Fig. 1c). Copper(II) ions produced by the photooxidation of copper(I) ions would have promoted the synthesis of cyanoacetylene (**6**) in the form of its solid-state copper(I) coordination compound, CuC_3N. Further addition of cyanide would have initiated the sequence of reactions that lead to **49** and **54**, the α-aminonitrile precursors of asparagine and aspartic acid, and glutamine and glutamic acid (Fig. 1d). Finally, synthesis of anhydronucleoside **7**, and thence the ribonucleotides **9** and **10**, could take place through the stream previously formed by the merger of two tributaries (containing the pentose aminooxazoline (**5**)) running into a pool that contained CuC_3N.

Conclusions

Although it necessarily has to be painted with broad brushstrokes, the picture that emerges is of an overall reaction network developing over time in separate streams and pools, according to a dynamic flow chemistry scheme. The various products would be synthesized by subtle variations in the flow-chemistry history of the streams and the order in which they merged or ran into pools. Although the overall scheme would not involve all the steps of the reaction network taking place simultaneously in ‘one pot’, the various products would end up mixed together in pools. Rather than invoking fundamentally different scenarios and chemistries for the syntheses of the molecular components of informational, compartment-forming and metabolic subsystems, and then concluding that one or other subsystem must have come first, we describe a scenario in which variations on a chemical homologation theme result in the components of all three subsystems being produced and then blended together. The reliance of the homologation chemistry on hydrogen cyanide (**11**) (all the carbon and nitrogen atoms in the compounds of the reaction network derive from this single source) and hydrogen sulfide (**12**) prompts us to use the term ‘cyanosulfidic’ to describe this protometabolic[38] systems chemistry.

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Author information

Affiliations

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK

Bhavesh H. Patel, Claudia Percivalle, Dougal J. Ritson, Colm D. Duffy & John D. Sutherland

Contributions

J.D.S. supervised the research and the other authors performed the experiments. All the authors contributed intellectually as the project unfolded. J.D.S. wrote the paper and B.H.P. and C.P. assembled the Supplementary Information, additionally incorporating data from D.J.R. and C.D.D.

Competing financial interests

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Corresponding author

Correspondence to: John D. Sutherland

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