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Reaching Back to Jump Forward: Recent Efforts towards a Systems-Level Hypothesis for an Early RNA World

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Experimental approaches toward discovering the chemical origins of life are often criticized for their lack of holistic guiding hypotheses; though justifiable, these assessments are mitigated somewhat by the general recognition that this is a really, really hard problem.

For the moment, let us set aside the challenges of demonstrating the template-catalyzed polymerization of an RNA replicator that avoids end-product inhibition, and focus solely on a potential prebiotic synthesis of a single pyrimidine nucleoside. The challenges inherent to the construction of just this monomer are formidable. The heterocyclic, saccharide, and phosphate chemical components of the monomer seemingly require multiple incompatible chemistries—and, by the way, these final building blocks do not react even when collected together.

However, this paradigm regarding the prebiotic synthesis of pyrimidine nucleosides has changed in the last few years, predominantly due to the efforts of the Sutherland group,^[1–6] which culminated in a recent paper in *Nature Chemistry*.^[7] The narrative of these discoveries begins with the work of a legendary figure in origins of life chemistry, Leslie Orgel, whose

group in 1970 published a remarkable synthesis of a cytidine nucleoside from ribose, cyanamide, and cyanoacetylene (Scheme 1 A).^[8,9] The direct condensation of ribose and a canonical pyrimidine heterocycle had never been observed under prebiotically plausible conditions (and still has not). However, Orgel's work side-stepped this difficult nucleosidation by closing the pyrimidine ring on a prebuilt ribose scaffold; interestingly, modern biology uses the same strategy.

However, from the perspective of jumping directly from small molecules to RNA monomers, this remarkable synthesis leaves some gaping holes. For example, how is ribose selected from the multitude of sugars produced from formaldehyde in a formose reaction? How is a continuous synthesis of nucleoside maintained when the reactants, cyanamide and cyanoacetylene, must be added sequentially, and without overlap, to this ribose? How is the primary α -cytidine product epimerized to the canonical β -cytidine (Scheme 1 A)?

The work of Sutherland's group has unified disparate issues within this prebiotic pyrimidine synthesis in a context that potentially connects pyrimidine formation with biologically relevant amino acid and glycerol syntheses. In 2009, the Suther-

Scheme 1. A) α -Cytidine is synthesized by treating ribose with cyanamide, followed by cyanoacetylene and then hydrolysis. β -Arabinocytidine and α -cytidine-5P are produced by replacing ribose with arabinose and ribose-5P, respectively (~10–30% yield of furanose anomers).^[8] B) The canonical β -cytidine is generated from arabinose-3P (~4% overall yield).^[1,10] C) The Sutherland group discovered that a selective source of pentoses was no longer necessary when the reaction was started with the small molecules glycolaldehyde and 2-amino-oxazole (glycolaldehyde + cyanamide; ~15% yield).

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land lab demonstrated a β -cytidine nucleotide synthesis from the simple sugar precursors glycolaldehyde (C₂) and glyceraldehyde (C₃), thus avoiding the necessity for a selective ribose synthesis (Scheme 1 C).^[3] The condensation of glycolaldehyde

with cyanamide produces 2-aminooxazole, which subsequently condenses with glyceraldehyde, and then cyanoacetylene to form the anhydro-arabino-nucleoside (I, Scheme 1C). Although dependent upon a specific order of reactant addition, each step in the synthesis of I proceeds in a mild aqueous environment and in remarkably good overall selectivity and yield. A regioselective phosphorylation of I is achieved by using ammonium phosphate suspended in heated urea/formamide. Spontaneous phosphorolysis generates the β -cytidine nucleotide containing an active cyclic phosphate diester (II, Scheme 1C).

Beginning the synthesis with C_2 and C_3 sugar fragments, rather than ribose, was a significant advance in demonstrating a plausible prebiotic synthesis of pyrimidine nucleosides. Subsequently, the Sutherland group sought to reach even further back to a sugar C_1 feedstock that is compatible with the HCN chemistry required for the generation of cyanoacetylene and cyanamide reactants.^[5–7] The oxidation of HCN to cyanoacetylene and cyanamide can be achieved by electric discharge in the gas phase or photo-oxidation in aqueous solution.^[9] HCN is also vital to numerous foundational syntheses in prebiotic chemistry including HCN polymerization to purines and the Strecker reaction to amino acids.

The reduction of HCN to the aldehydic oxidation state required for sugar chemistry is challenging; the reductant must be able to form, but not over-reduce, the imine/aldehyde product. The Sutherland group settled on a disproportionation of HCN catalyzed by a light-activated Cu^I complex (Scheme 2A).^[5] The products of the disproportionation are formaldehyde and isocyanic acid. Subsequent reaction of the formaldehyde with cyanide generates cyanohydrin, which is reduced and hydrolyzed to glycolaldehyde, a C_2 sugar (Scheme 2B). This procedure of cyanide attack followed by re-

duction and hydrolysis (a Kiliani–Fischer synthesis) can be repeated to produce glyceraldehyde, a C_3 sugar, from glycolaldehyde. Both sugars were detected as their oxazolidinones, which were produced by spontaneous condensation of the aldehydes with isocyanic acid (Scheme 2C). These oxazolidinones are not productive in pyrimidine synthesis, and thus research efforts were begun to find a prebiotically plausible sacrificial reductant that would avoid isocyanic acid formation, and the resulting irreversible glycol- and glyceraldehyde sequestration.^[6,7] Hydrogen sulfide was found to be both a suitable and plausible reagent.

The reaction of hydrogen sulfide with cyanohydrin in the presence of catalytic Cu^I produces glycolaldehyde in over 40% yield (Scheme 3A).^[6] As oxazolidinone formation is prevented, the generation of other carbonyl compounds such as glyceraldehyde (by reductive homologation of glycolaldehyde, Scheme 3B) as well as acetaldehyde and formaldehyde (by reduction of glycolaldehyde) becomes more evident. Interestingly, subsequent addition of 1 equiv of cyanide and excess ammonia at pH 9 slowly produces the nitrile analogues of serine, glycine, and alanine from glycolaldehyde, formaldehyde, and acetaldehyde, respectively (Scheme 3C). Additionally, the aminonitriles are more resistant to reduction than the cyanohydrins, thus providing a rationale for the generation of nucleotides from the reduction of cyanohydrins ($NC-CH_2-OH$) and amino acids from the hydration of aminonitriles ($NC-CH_2-NH_2$). These results potentially link amino acid and nucleoside synthesis to common building blocks and a common environment.

In their most recent work,^[7] the Sutherland group has shown that glyceraldehyde, produced from the reductive homologation of glycolaldehyde, tautomerizes to dihydroxyacetone in 60% yield in phosphate buffer (Scheme 4). Reduction of dihydroxyacetone produces both acetone and glycerol, again in high yields. Glycerol was quantitatively phosphorylated into a mixture of phosphate isomers by heating with ammonium phosphate in urea/formamide. Glycerol phosphates are the fatty ester/ether linkers required for membrane synthesis across all of biology, and represent another cog in a poten-

Scheme 2. A) An irradiated (254 nm) copper(I)-catalyzed disproportionation of hydrogen cyanide produces formaldehyde and isocyanic acid. B) Reductive homologation of formaldehyde and glycolaldehyde with cyanide generates glycolaldehyde (15%) and glyceraldehyde (1–5%), respectively. C) Glycolaldehyde and glyceraldehyde are trapped by isocyanic acid as oxazolidinones.

Scheme 3. A) The reduction of cyanohydrin produces glycolaldehyde in 42% yield (after 2 h), and acetaldehyde in 15% yield (after 6 h). B) Glyceraldehyde is produced in 17% yield by the reductive homologation of glycolaldehyde. C) The aminonitrile precursors of amino acids are generated from the nucleophilic addition of cyanide to an imine.

Nucleotide chemistry:

Peptide chemistry:

Lipid chemistry:

Scheme 5. The synthesis of pyrimidine nucleotides, amino acids, and glycerol phosphate from cyanohydrin. The following reaction types are employed: 1) Reduction with NaSH, cat. Cu^I, and irradiation at 254 nm, 2) reaction with cyanamide (potentially sourced from calcium ferrocyanide), 3) reaction with cyanoacetylene (potentially sourced from HCN and calcium carbide), 4) dehydrative phosphorylation, accomplished experimentally with inorganic phosphate in hot urea/formamide, and 5) Strecker syntheses with HCN and ammonia (potentially sourced from the hydration of magnesium nitride).

Scheme 4. Glyceraldehyde at RT in pH 8 phosphate buffer tautomerizes to dihydroxyacetone in ~60% yield after 20 days. Reduction of dihydroxyacetone generates glycerol in ~35% yield and acetone in ~30% yield. Phosphorylation with ammonium phosphate at 120 °C for 7 days produces glyceraldehyde phosphate isomers in near quantitative yield. a) NaSH, 10 mol % CuCN, $h\nu$ (254 nm); b) HOPO₃²⁻ in urea/formamide.

tial systems-level hypothesis for the origin of multiple biological polymers from a single source. The acetone also provides an entry into valine and leucine amino acid syntheses through two and three, respectively, reductive homologations with cyanide and sodium sulfide.

The Sutherland group has proposed a scenario to link three major prebiotic syntheses back to a common environment containing cyanide and hydrogen sulfide. Experimental verification of the scenario demonstrates a succession of selective, high-yielding reactions. The overall scheme still requires multiple incompatible chemistries (arguably 1–5, Scheme 5) that require separation prior to pooling. A scenario is described^[7] in which streams, fed by rain and mineral deposits, might have

allowed this isolated chemistry to occur before recombination. Though this argument is not, on its surface, as “aesthetic” as the field might hope, it still represents the most holistic, experimentally supported hypothesis on the origins of an RNA world directly from small building blocks.

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