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RNA World

Evolution. A theory of change through time that seeks to explain both the origins of life and the path life has taken to reach the diverse nature of today. The core concepts of fitness and species competition introduced by Darwin over a hundred and forty years ago lay the foundation for evolutionary thought. By these rules the true processes of selection brought about by competition and survival among the fittest have been validated time and time again. However, one problem remains. At the core of evolutionary thought lies the concept of heredity—the ability to acquire changes and pass them through the eons of time. In 1953 the structure of DNA was discovered (Garrett and Grisham, 1999) and with it came the validation that the nucleic acid truly is the vehicle that drives evolutionary change. If evolution began with *de novo* formation of nucleic acids, as most scientists believe it did (Orgel, 2004), then evolutionary theory rests on the capacity to prove that such *de novo* formation was feasible during early earth evolution.

The nucleic acid is the cornerstone of evolutionary theory, and it is therefore the purpose of this paper to investigate the proposed first steps that may have led to nucleic acid formation. The predominating theory now revolves around a concept known as the RNA world (Joyce , 1989) in which early RNA molecules possessed all of the functional characteristics necessary for information transfer through time. In this theory, the DNA/RNA/protein interactions that drive all living processes on earth today likely arose

first from the initial formation of RNA early in the prebiotic world (environment comprised of chemicals likely present during early earth evolution). However, it should not be misconstrued that RNA formation was necessarily an autonomous process. Many studies are challenging this concept, proposing that RNA evolution more than likely occurred through a systematic process of pre-RNA intermediates (Joyce, 1989). This is a valid and important point that will be discussed, and yet it should be noted that the predominant scope of this paper is to determine the plausibility of RNA formation under prebiotic conditions. For this reason, we will focus on the most relevant concern of nucleic acid formation by investigating the leading theories of RNA constituent synthesis in prebiotic conditions, as well as how the enzymatic characteristics of RNA have led to the postulation of its feasibility as the precursor to the DNA/RNA/protein era of today.

RNA Structure Review

An adequate review of the postulated processes of RNA formation requires a brief review of the make-up of the RNA molecule itself. RNA is a complex polymer composed of repeating units known as nucleotides. These nucleotides are each comprised of a phosphate backbone, pentose sugar, and an alternating nitrogenous base, which together provide the specificity and informational encoding character of the molecule (Garrett & Grisham, 1999). This incredibly complex molecule composed of multiple complex parts has driven most scientists away from hypothesizing a one-step, *de novo* mechanism for the formation of the entire RNA molecule. Instead, they have sought to first hypothesize how each constituent part of RNA—the phosphate backbone, pentose sugar, and nitrogenous base—could have formed autonomously and then lead to

the final molecular integration of the three constituents (Orgel, 2004). This paper will also follow the same general layout of RNA development and to that direction we now turn.

Non-enzymatic formation of RNA Constituents

According to Orgel's 2004 review paper on RNA origins, the study of RNA formation must follow a presumed set of criteria. Prebiotic chemistry deals with molecules hypothesized to be present and capable of formation during the ancient years of early earth formation. Thus, Orgel argues that all hypotheses for molecular synthesis must deal only with chemical reactions that employ chemicals and compounds that could have been present in "adequate" amounts during the prebiotic era. In addition, reactions must occur in water, due to the postulated water environment of early earth. Finally, the intended products must also be in "significant" amounts to merit the plausibility of hypothesized synthetic reactions that could have lead to proposed RNA constituent formation. These criteria will thus be assumed whenever "prebiotic" conditions are referred to.

In addition, the analysis of a great deal of the hypothesized synthetic pathways involves detailed analysis of organic chemistry mechanisms. For the purpose of this paper, no detailed mechanistic elements will be analyzed—only the major organic reactions comprising the synthesis in a non-enzymatic environment will be investigated. Non-enzymatic analysis is also a key point that should not be overlooked. In modern biology, almost all *in vivo* enzymatic reactions connote protein catalysis, whereas in the prebiotic world, no catalytic proteins likely existed (Orgel, 2004). Though some

scientists would likely contest this presupposition, this analysis will follow the same assumption and investigate only reactions that can proceed in a non-catalytic environment.

RNA Constituent Synthesis

The first component of RNA to be analyzed is the hypothesized formation of pentose sugars. The major reaction studied thus far involves the conversion of Formaldehyde to a variety of complex, biochemical sugars in an aqueous environment (Mizuno & Weiss, 1974 as cited in Orgel, 2004). The process involves an autocatalytic reaction that initially produces glycolaldehyde—a product which is later converted to glyceraldehydes as well as a variety of other four, five, and six carbon sugars. The progressive investigation of this reaction has led to the discovery of the production of a variety of relevant biomolecules including ribose sugars that sometimes contain phosphate linkages. Though the validity of this process has been challenged due to the necessity for high concentrations of reactant that yield very low levels of ribose, recent experiments have bolstered this hypothesis by revealing significant yields through the addition of inorganic catalysts (Pitsch *et al*., 1995 as cited in Orgel, 2004). Overall, experiments to date reveal that the formation of the ribose component of RNA is plausible within environmental constraints of prebotic evolutionary analysis.

The next analysis focuses on the *de novo* formation of purine nitrogenous bases. Again, the reactions studied thus far for purine formation involve complex mechanistic reactions. Only a broad overview of the major players involved in this process and the relevant products formed will be discussed. Between the nitrogenous base sets, purines

have proven to be the more difficult base set to form under prebiotic constraints. The major set of reactions studied thus far involves the polymerization reaction of HCN in aqueous solution leading to an HCN tetramer (Ferris & Orgel, 1965, 1966a; Sanchez *et al.*, 1967, 1968 as cited in Orgel, 2004). This tetramer can then react with AICN (a product of HCN and ammonia reaction) to form adenine—one of two purine bases composing RNA. However, a problem arises with the plausibility of this process. HCN is a highly volatile molecule and thus HCN concentrations required for this reaction likely could not have formed within the prebiotic ocean. However, experiments involving HCN concentration through freezing have thwarted this problem. Through a process known as "eutectic freezing", water crystals settle out of solution, super concentrating the HCN that remains (Sanchez *et al.*, 1966a as cited in Orgel, 2004). This process coupled to the AICN reaction provides a likely pathway for prebiotic adenine synthesis (Orgel, 2004).

The road to pyrimidine synthesis within the context of prebiotic constraints is a short one when compared to the purine synthetic path just traversed. Most of the reactions involve a reaction between cyanoacetylene and cynate ions, cyanogens, or urea to produce cytosine in good yield (Ferris *et al.*, 1968, 1974; Robertson & Miller, 1995a, 1995b; Nelson *et al.*, 2001 as cited in Orgel, 2004). This reaction fits within the prebiotic constraints because cyanoacetylene is a major product formed when electricity is passed through nitrogen and methane mixtures (Sanchez *et al.*, 1966b as cited in Orgel, 2004). The cytosine produced via this reaction can also be easily converted to uracil via simple hydrolysis. The eutectic freezing model has also been employed with this reaction,

producing higher yields of cytosine and proposing a process that links both purine and pyrimidine synthesis (Orgel, 2004).

The successful *in vitro* synthesis of ribose and the nucleoside bases paves the first steps in the path of the RNA formation. However, it is the process of melding these components to yield nucleotides which raises another hurtle for plausible RNA evolution under prebiotic constraints. Pyrimidine nucleoside formation from the direct reaction of cytosine or uracil and ribose has never been shown (Sanchez & Orgel, 1970 as cited in Orgel, 2004). However, several researchers have obtained nucleoside formation via multiple step synthetic reactions involving the intermediate, arabinose-3'-phosphate. The plausibility of this intermediate being present during prebiotic conditions is still not clear; however, it is believed to be a potential mechanism for nucleoside formation during early earth evolution (Orgel, 2004).

The final step in RNA synthesis involves the attachment of the phosphate group to the nucleoside subunits to create nucleoside monophosphates. Many types of reactions have been explored to identify how nucleosides could be phosphorylated under prebiotic conditions. One of the major reactions yielding mono or polyphosphate nucleosides involves the reaction of nucleosides with ammonium phosphate and urea (Lohrmann & Orgel, 1971 as cited in Orgel, 2004). These reactions do yield nucleotides, but with multiple phosphate linkages along the ribose sugar. Attempts have also been made to directly phosphorylate the normal 5' position of the ribose sugar, and some success has been made (Handschuh *et al.*, 1973; Osterberg *et al,*. 1973; Reimann & Zubay, 1999 as cited in Orgel, 2004).

Ultimately, a review of RNA constituent synthesis under prebiotic constraints reveals plausible routes for RNA constituent formation. Though not conclusive, research thus far does support the validity that RNA components could have likely formed during early earth evolution.

RNA Polymerization

The process of RNA constituent formation is indeed one the greatest hurtles for evolutionary biologists to overcome. As illustrated in the previous section, though difficult, researchers have taken great strides toward proving the plausibility of RNA formation in the prebiotic world. Once RNA monomers formed, the next leap involves hypothesizing how RNA monomers could have polymerized to yield the informational encoding character of the RNA molecule. It is this topic that brings us to a fork in the road regarding RNA world research. In one direction, we encounter a hypothesis supporting a *direct* polymerization event leading to RNA monomeric integration, whereas the other path introduces an *indirect* synthesis leading to the same end. The next section will examine the validity of a *direct* RNA polymerization event by analyzing what evidence supports such a mechanism.

The attempt to polymerize nucleotides in aqueous solution has been met with some level of difficulty. The synthesis of any polymer from monomers is an endergonic process, requiring an input of energy from an external source (Garrett & Grisham, 1999). In 1967, Moravek showed that evaporating solutions containing mixtures of nucleotides followed by heating could provide enough energy to produce very short oligonucleotides with 2'-5' and 3-'5' linkages (Orgel, 2004). However, due to the short nature and

mixture of linkage orientations produced by this reaction, other avenues for energy input were sought after. Pre-activation of the nucleotides was thought to be a better approach, and yet even nucleoside-5'-polyphosphates react very slowly in aqueous environments (Orgel, 2004). This ultimately led to the activation via phophoramidates. Such activated monomers are accepted as prebiotic because they can be obtained from the conversion of nucleoside-5'-polyphosphates (Orgel, 2004). The first class of reactions involving these activated nucleoside 5'-phosphorimidazolides revealed the production of long homo and hetero-oligomers when lead ions were used in conjunction with eutectic freezing (Kanavarioti *et al.*, 2001; Monnard *et al.*, 2003 as cited in Orgel, 2004). The products were, however, predominantly 2'-5' linked instead of the desired 3'-5' linkages comprising modern RNA.

This problem of multiple linkage orientations has potentially been solved, however, by the identification of a polymer clay known as montmorillonite. This clay has shown to both catalyze phosphodiester linkages as well as direct linkages in the "proper" 3'-5' orientation. The structure and catalytic activity of these clay catalysts have been extensively studied, revealing activity under even low nucleoside concentrations and yielding polymerization of oligomers up to 50 residues with 3'-5' phosphodiester linkages comprising approximately 80% of the reaction product (Huang & Ferris, 2003; Prabahar & Ferris, 1997 as cited in Orgel, 2004). Though extensive details of these findings will not be discussed, the relevance of clay catalysis on RNA polymerization through increased efficiency of reactions and enhanced polymer length are incredibly significant to the validation of RNA polymerization plausibility in a prebiotic setting.

Template Directed Synthesis

The successful polymerization of RNA leads to the information encoding character of the molecule and yet such information content is useless if it cannot be faithfully passed onto following RNA "generations". This need for a replication mechanism that does not rely on protein catalysis is where scientists next turned. Howard *et al.* in 1966 showed that incubation of a polynucleotide with mononucleotides could develop into double and triple helical structures, resembling common-day nucleic acids. The reaction naturally incorporated complementary bases, but with a drastic flaw. Again, the linkages were comprised of a complex mixture of 2'-5' and 3'-5', and thus scientists needed a find a mechanisms that could replicate with predominantly 3'-5' helical orientations. This search led to the regiospecific polymerization reaction that involved pre-activated guanosine-5'-phosphorimidazolide as monomers replicating a poly-cytosine template. When magnesium and zinc ions were added to the reaction, almost pure 3'-5-linked helices formed (Bridson and Orgel, 1980 as cited in Orgel, 2004). As discussed above, the catalytic activity of montmorillonite clays also has been shown to preferentially catalyze 3'-5' linkages, further disarming this linkage orientation issue, and strengthening the plausibility of early RNA polymer replication.

The "success" of these replicating experiments does indeed support aspects of the RNA world hypothesis, and yet there exists several significant flaws within all such studies. As explained in Joyce's 1984 paper, any reactions involving polynucleotide formation would yield racemic mixtures of L-enantiomeric and D-enantiomeric configurations. Successful polymerization and incorporation of D-monomoers (the predominant form on earth today) is greatly inhibited by the presence of L-configured

monomers, introducing a very large question (Schwartz, 1998). How did early RNA replication occur if all hypothesized RNA synthetic reactions involved racemic mixtures that would consequently inhibit the successful replication of RNA populations?

Some scientists believe the racemic replication problem could be solved through complex mechanisms again involving catalytic clays. Through a coordinated interplay between template-directed syntheses of oligomers on clay surfaces in conjunction with metal ions, greater regiospecificity could have possibly arisen, thus dispelling the racemic inhibition response during replication (Ferris, 1993 as cited in Ertem and Ferris, 1996). Many scientists, however, do not believe that such a scenario is adequate to overcome the insurmountable problem of racemic inhibition. This has forced many scientists to rethink the evolutionary path of RNA formation in a way that can side step this large chasm introduced by racemic complication. It is toward this new direction of *indirect* RNA integration that we now turn.

A pre-RNA world

Due to the incredible hurtles that racemic products present to feasible RNA polymerization, many researchers have moved to a different camp that embraces an indirect mechanism of RNA polymerization—a concept known as the pre-RNA world hypothesis (Joyce *et al.*, 1987 as cited in Schwartz, 1998). This hypothesis revolves around the fact that the inevitable inhibition of RNA polymerization due to enantiomeric contamination could be overcome by introducing a simpler, achiral genetic system that is not compromised by racemic complications. This achiral, genetic material could then evolve through time to integrate aspects of RNA in a manner that would "naturally"

select for specific racemates, consequently dispelling the problem with racemic inhibition. Joyce presents this hypothesis with a list of four requirements for the pre-RNA genetic material. It must: 1) be able to convey information via at least 2 monomeric subunits comprising a heteropolymer; 2) be able to direct the ordered assemblage of monomeric units to create additional copies of itself (self-replication); 3) have substantial concentrations of subunits available for reactions to proceed; 4) and have stability profiles that ensure that the rate of production exceeds the rate of degradation (Joyce, 1989). But is this concept simply a stab in the dark, or does science support such a hypothesis?

There is actually considerable evidence that a pre-cursor to RNA likely first arose. One of the most notable systems studied thus far is referred to as "Peptide Nucleic Acid" or PNA. This polymer is composed of purines and pyrimidines attached via a triagonal nitrogen atom, providing a structure with truly achiral character (Wittung *et al*., 1994 as cited in Schwartz, 1998). Such composition allows the polymer to form either right-handed or left-handed helices, thus providing a system potentially devoid of racemic complications (Schwartz, 1998). In addition, this polymer has been shown to form self Watson-Crick base pairing as well as non-self pairing with RNA nucleotides. Taken together, these findings provide credence to the plausibility of a pre-RNA to RNA transition through some nucleic acid intermediate (Shoning *et al.*, as cited in Joyce, 1989).

If such a precursor to RNA was present, then there must exist a mechanism to transition from the pre-RNA polymer to RNA molecules that would later author in an RNA dominated world. Joyce offers several scenarios for this transition, depending on

the nature of the pre-RNA molecule itself. If the molecule was capable of RNA cross pairing, then the gradual transition could be compared to transcriptional evolution, where pre-RNA monomers were gradually replaced with RNA nucleotides during successive rounds of replication. Such an event would likely occur because the incorporation of RNA nucleotides would yield some advantage to the pre-RNA/RNA complex, slowly favoring the RNA constituents, and eventually yielding a polymer solely comprised of RNA. If instead the pre-RNA molecule was not capable of RNA cross-pairing, then a possible "translational" event could have occurred, leading to the development of RNA polymerization and consequent replacement through selection (Joyce, 2002).

Ribozymes—RNA Catalysts

The mechanism by which RNA mononucleotides may have polymerized is still in question, and yet the fact that it happened is not. Yet, initial and spontaneous polymerization events are useless to prebiotic evolution if replication of these polymers could not occur. Replication experiments reviewed earlier do provide some evidence to the potential for non-enzymatic replication, and yet the problems with racemic inhibition and incredibly slow rates of nucleotide incorporation do not facilitate a mechanism for "significant" chemical/RNA evolution. At some point catalytic replication would have to occur for chemical evolution to proceed. Amazingly enough, this problem with RNA polymer replication is being answered by the mere presence of RNA polymers, themselves. Studies are indicating that as polymers of RNA began to accumulate, whether through direct RNA mononucleotide assimilation or via indirect integration through a pre-RNA intermediate, a new world of reactions began (Joyce, 2002).

Suddenly the prebiotic pools were filling with molecules that possessed catalytic activity. The ribozyme was born. Postulation of how this process initially began and developed has consequently led to some of the most exciting discoveries involving RNA world research.

Evidence of ribozymes exist all around us, with one of the greatest examples found in our own bodies, catalyzing one of the most import anabolic reactions known to mankind. This act of translation is facilitated by the activity of a subcelluar structure known as the ribosome (Garrett & Grisham, 1999). The ribosome is composed of a complex mixture of protein and ribosomal subunits, and it has been shown that the peptide-forming step of protein synthesis involves direct interaction of the catalytic RNA subunit (Garrett & Grisham, 1999; Steitz & Moore, 2003 as cited in Orgel, 2004). This finding as well as a vast body of ribosomal research reveals that RNA sequences can indeed catalyze reactions, and if they are capable of catalysis today, we should wonder what reactions they were capable of during prebiotic evolution.

Ribozymes present in the prebiotic world were very likely to possess catalytic activity, and as studies have sought to determine what types of reactions these early ribozymes took part in, they have consequently revealed ribozymal function in a vast array of diverse anabolic and catabolic reactions. The ultimate reaction of interest, however, is the reaction involving RNA replication—are ribozymes capable of performing template directed synthesis? Research points to yes. In a process referred to as “continuous *in vitro* evolution” scientists are using PCR technology to simulate early RNA evolution via replication, and through the process are finding that early pools of

polymerized RNA could have likely evolved through chemical selection based on specific catalytic activity (Orgel, 2004).

In one hallmark study, scientists began with a catalytic RNA known as the class I ligase. This initial ribozyme was approximately 120 base pairs in length, and possessed minimal RNA-dependent-RNA polymerase activity, evidenced by its capacity to add three residues to the 3' end of an RNA primer template (Ekland *et al.,* 1996 as cited in Joyce, 2002). During successive rounds of *in vitro* evolution, however, the final ribozyme "product" contained approximately 200 nucleotides and was capable of adding up to 14 nucleotides in a 24 hour period, with 97% fidelity (Johnston *et al.,* 2001 as cited in Joyce, 2002). Though capable of only minimal replication (addition of only 14 residues), these experiments have given significant credence to the possibility of RNA polymeric evolution and consequent catalytic function necessary for RNA replication.

Additional studies investigating *in vitro* RNA evolution have bolstered this hypothesis by also revealing that ribozyme-like sequences could have formed from random sequence pools. One particular study has shown that through successive rounds of *in vitro* selection and amplification, unique RNA ligase ribozymes with dual activities have been generated. These results provide strong evidence that complex ribozymal structures could have originated from relatively simple, short existing sequences. With ample time and multiple rounds of breaking and adjoining of random sequences, the production of complex, functional ribozymes would be inevitable (Landweber and Pokrovskaya, 1999).

A final and important note comes again from Orgel's 2002 paper, where she argues that there still exist major holes with the ribozyme evolution hypothesis.

Replication events via ribozymal catalysis have been shown to replicate templates up to only 14 residues in length. How could a fully functional ribozyme evolve if catalytic activities permit only replication of a fraction of the ribozyme sequence? In addition, ribozymes must also be capable of separating the double-stranded product after replication. No known ribozyme has been found to possess this unique catalytic activity, and without strand separation, successive rounds of replication would be impossible and RNA evolution could not have occurred (Orgel, 2002). In rebuttal, one should not forget evidence provided in the aforementioned ribozyme research. If scientists are "creating" RNA polymers capable of partial self-replication, one could argue that with ample rounds of replication, catalytic processes capable of total self-replication as well as strand-separation could also be possible. Though no known studies have arisen to *directly* support the validity of this argument, one could agree that the dynamics of this process lie well within the constraints of RNA formation hypotheses to date.

Where Should We Stand?

The task to accurately map the evolutionary path from simple, organic molecules to complex polymers capable of self-replication is difficult—especially when the path itself lies under several billion years of chemical ambiguity. After decades of RNA evolutionary research, many critical questions remain unanswered, and yet alongside them, many others have been solved. Despite the challenges and major gaps that remain unfilled, scientists have successfully reconstructed many possible mechanisms for RNA evolution. By outlining many of the reactions necessary to produce RNA constituents and investigating a multitude of different scenarios that could have led to RNA formation

and polymerization, we now have a far clearer picture regarding the processes necessary to invoke successful RNA formation. The question of whether an intermediate nucleic acid derivative authored in the RNA era or whether RNA evolved autonomously is still in question, and only further research will bring light to this issue. Polymers did form, however, and the latest research on ribozyme structure and function is revealing a molecule with the plasticity and catalytic capability to accelerate replication and begin the accumulation of genetic change.

Evolution—the process of accumulated genetic change through time. Science long ago unveiled the vehicle responsible for such change and we are now left to answer how this vehicle itself first formed. We have learned a great deal regarding how such an event could have occurred, and as we seek to fill in the gaps, more and more will undoubtedly be uncovered. The world of RNA evolutionary research attempts to reveal mechanisms that occurred eons ago, and we must realize this goal may never fully be met. Yet, above all we must seek to answer the most important question. Could such a process have ever actually occurred? In a review article of nucleic acid structure, Dr. Albert Eschenmoser reiterates this claim with his belief that, " Eventually, the course and the outcome of such studies would emphasize the point that…chemistry must be not primarily to show how life on Earth could have originated, but to provide decisive experimental evidence—through the realization of model systems in the laboratory ("artificial chemical life")—that life can arise as a result of the organization of organic matter" (Eschenmoser, 1999). Evidence supports the notion that RNA truly is one of the oldest and greatest ancestors of our past, surviving and evolving through the eons of time and representing a true relic of evolutionary processes. The map of RNA evolution is not

finished, and yet for anyone who believes in the power of chance must also have faith that with ample time, the building blocks of life would emerge—building blocks that are thought to have shaped and formed organismal existence and ultimately led us, as humans, to contemplate how it all began. In the end, however, we are left to decide for ourselves what we believe to be true.

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