The Origins of the RNA World

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SUMMARY

The general notion of an "RNA World" is that, in the early development of life on the Earth, genetic continuity was assured by the replication of RNA and genetically encoded proteins were not involved as catalysts. There is now strong evidence indicating that an RNA World did indeed exist before DNA- and protein-based life. However, arguments regarding whether life on Earth began with RNA are more tenuous. It might be imagined that all of the components of RNA were available in some prebiotic pool, and that these components assembled into replicating, evolving polynucleotides without the prior existence of any evolved macromolecules. A thorough consideration of this "RNA-first" view of the origin of life must reconcile concerns regarding the intractable mixtures that are obtained in experiments designed to simulate the chemistry of the primitive Earth. Perhaps these concerns will eventually be resolved, and recent experimental findings provide some reason for optimism. However, the problem of the origin of the RNA World is far from being solved, and it is fruitful to consider the alternative possibility that RNA was preceded by some other replicating, evolving molecule, just as DNA and proteins were preceded by RNA.

Outline

Cold Spring Harbor Perspectives in Biology

1 INTRODUCTION

The general idea that, in the development of life on the Earth, evolution based on RNA replication preceded the appearance of protein synthesis was first proposed over 40 yr ago (Woese 1967; Crick 1968; Orgel 1968). It was suggested that catalysts made entirely of RNA are likely to have been important at this early stage in the evolution of life, but the possibility that RNA catalysts might still be present in contemporary organisms was overlooked. The unanticipated discovery of ribozymes (Kruger et al. 1982; Guerrier-Takada et al. 1983) initiated extensive discussion of the role of RNA in the origins of life (Sharp 1985; Pace and Marsh 1985; Lewin 1986) and led to the coining of the phrase "the RNA World" (Gilbert 1986).

"The RNA World" means different things to different investigators, so it would be futile to attempt a restrictive definition. All RNA World hypotheses include three basic assumptions: (1) At some time in the evolution of life, genetic continuity was assured by the replication of RNA; (2) Watson-Crick base-pairing was the key to replication; (3) genetically encoded proteins were not involved as catalysts. RNA World hypotheses differ in what they assume about life that may have preceded the RNA World, about the metabolic complexity of the RNA World, and about the role of small-molecule cofactors, possibly including peptides, in the chemistry of the RNA World.

There is now strong evidence indicating that an RNA World did indeed exist on the early Earth. The smoking gun is seen in the structure of the contemporary ribosome (Ban et al. 2000; Wimberly et al. 2000; Yusupov et al. 2001). The active site for peptide-bond formation lies deep within a central core of RNA, whereas proteins decorate the outside of this RNA core and insert narrow fingers into it. No amino acid side chain comes within 18 Å of the active site (Nissen et al. 2000). Clearly, the ribosome is a ribozyme (Steitz and Moore 2003), and it is hard to avoid the conclusion that, as suggested by Crick, "the primitive ribosome could have been made entirely of RNA" (1968).

A more tenuous argument can be made regarding whether life on Earth began with RNA. In what has been referred to as "The Molecular Biologist's Dream" (Joyce and Orgel 1993), one might imagine that all of the components of RNA were available in some prebiotic pool, and that these components could have assembled into replicating, evolving polynucleotides without the prior existence of any evolved macromolecules. However, a thorough consideration of this "RNA-first" view of the origin of life inevitably triggers "The Prebiotic Chemist's Nightmare", with visions of the intractable mixtures that are obtained in experiments designed to simulate the chemistry of the primitive Earth. Perhaps this continuing nightmare will eventually have a happy ending,

and recent experimental findings provide some reason for optimism. However, the problem of the origin of the RNA World is far from being solved, and it is fruitful to consider the alternative possibility that RNA was preceded by some other replicating, evolving molecule, just as DNA and proteins were preceded by RNA.

2 AN "RNA-FIRST" VIEW OF THE ORIGIN OF LIFE

2.1 Abiotic Synthesis of Polynucleotides

This section considers the synthesis of oligonucleotides from ß-D-nucleoside 5′-phosphates, leaving aside for now the question of how the nucleotides became available on the primitive Earth. Two fundamentally different chemical reactions are involved. First, the nucleotide must be converted to an activated derivative, for example, a nucleoside 5′-polyphosphate. Next the 3′-hydroxyl group of a nucleotide or oligonucleotide molecule must be made to react with the activated phosphate group of a monomer. Synthesis of oligonucleotides from nucleoside 3′-phosphates will not be discussed because activated nucleoside 2′- or 3′-phosphates in general react readily to form 2′,3′-cyclic phosphates. These cyclic phosphates are unlikely to oligomerize efficiently because the equilibrium constant for dimer formation is only of the order of 1.0 L/mol (Erman and Hammes 1966; Mohr and Thach 1969). In the presence of a complementary template somewhat larger oligomers might be formed because the free energy of hybridization would help to drive forward the chain extension reaction.

In enzymatic RNA and DNA synthesis, the nucleoside 5′-triphosphates (NTPs) are the substrates of polymerization. Polynucleotide phosphorylase, although it is a degradative enzyme in nature, can be used to synthesize oligonucleotides from nucleoside 5′-diphosphates. Nucleoside 5′-polyphosphates are, therefore, obvious candidates for the activated forms of nucleotides. Although nucleoside 5′-triphosphates are not formed readily, the synthesis of nucleoside 5′-tetraphosphates from nucleotides and inorganic trimetaphosphate provides a reasonably plausible prebiotic route to activated nucleotides (Lohrmann 1975). Other more or less plausible prebiotic syntheses of nucleoside 5′-polyphosphates from nucleotides have also been reported (Handschuh et al. 1973; Osterberg et al. 1973; Reimann and Zubay 1999). Less clear, however, is how the first phosphate would have been mobilized to convert the nucleosides to 5′-nucleotides. Nucleoside 5′-polyphosphates are high-energy phosphate esters, but are relatively unreactive in aqueous solution. This may be advantageous for enzyme-catalyzed polymerization, but is a severe obstacle for the nonenzymatic polymerization of nucleoside 5′-polyphosphates, which would occur far more slowly than the hydrolysis of the resulting polynucleotide.

In a different approach to the activation of nucleotides, the isolation of an activated intermediate is avoided by using a condensing agent such as a carbodiimide (Khorana 1961). This is a popular method in organic synthesis, but its application to prebiotic chemistry is problematic. Potentially prebiotic molecules such as cyanamide and cyanoacetylene activate nucleotides in aqueous solution, but the subsequent condensation reactions are inefficient (Lohrmann and Orgel 1973).

Most attempts to study nonenzymatic polymerization of nucleotides in the context of prebiotic chemistry have used nucleoside 5′-phosphoramidates, particularly nucleoside 5′-phosphorimidazolides. Although phosphorimidazolides can be formed from imidazoles and nucleoside 5′-polyphosphates (Lohrmann 1977), they are only marginally plausible as prebiotic molecules. They were chosen because they are prepared easily and react at a convenient rate in aqueous solution.

Nucleotides contain three principal nucleophilic groups: the 5′-phosphate, the 2′-hydroxyl, and the 3′-hydroxyl group, in order of decreasing reactivity. The reaction of a nucleotide or oligonucleotide with an activated nucleotide, therefore, normally yields 5′,5′-pyrophosphate-, 2′,5′-phosphodiester-,

and 3′,5′-phosphodiester-linked adducts (Fig. 1A), in order of decreasing abundance (Sulston et al. 1968). Thus the condensation of several monomers would likely yield an oligomer containing one pyrophosphate and a preponderance of 2′,5′-phosphodiester linkages (Fig. 1B). There is little chance of producing entirely 3′,5′-linked oligomers from activated nucleotides unless a catalyst can be found that increases the proportion of 3′,5′-phosphodiester linkages. Several metal ions, particularly Pb^{2+} and UO_2^{2+}, catalyze the formation of oligomers from nucleoside 5′-phosphorimidazolides (Sleeper and Orgel 1979; Sawai et al. 1988). The Pb^{2+}-catalyzed reaction is especially efficient when performed in eutectic solutions of the activated monomers (in concentrated solutions obtained by partial freezing of more dilute solutions). Substantial amounts of long oligomers are formed under eutectic conditions, but the product oligomers always contain a large proportion of 2′,5′-linkages (Kanavarioti et al. 2001; Monnard et al. 2003).

What kinds of prebiotically plausible catalysts might lead to the production of 3′,5′-linked oligonucleotides directly from nucleoside 5′-phosphorimidazolides or other activated nucleotides? It is unlikely, but not impossible, that a metal ion or simple acid-base catalyst would provide
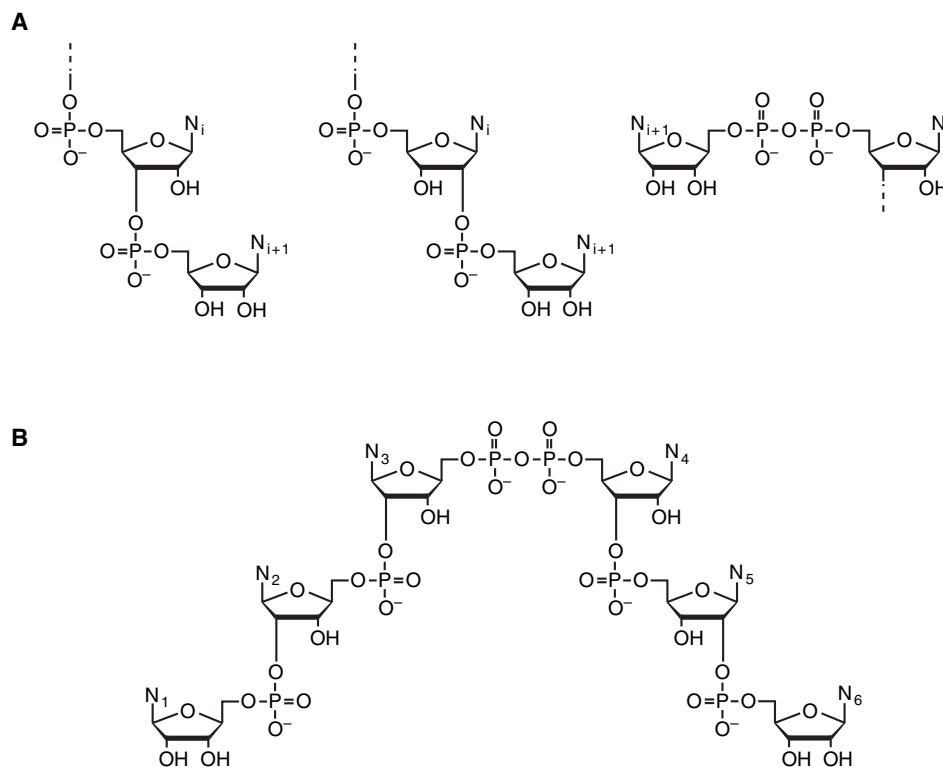
Figure 1. Phosphodiester linkages resulting from chemical condensation of nucleotides. (A) Reaction of an activated mononucleotide (N_{i+1}) with an oligonucleotide ($N_1–N_i$) to form a 3′,5′-phosphodiester (left), 2′,5′-phosphodiester (middle), or 5′,5′-pyrophosphate linkage (right). (B) Typical oligomeric product resulting from chemical condensation of activated mononucleotides.

sufficient regiospecificity. The most attractive of the other hypotheses is that adsorption to a specific surface of a mineral might orient activated nucleotides rigidly and thus catalyze a highly regiospecific reaction.

The work of Ferris and coworkers provides support for this hypothesis (Ferris et al. 2004; Ferris 2006). They have studied the oligomerization of nucleoside 5′-phosphorimidazolides and related activated nucleotides on the clay mineral montmorillonite (Ferris and Ertem 1993; Kawamura and Ferris 1994; Miyakawa and Ferris 2003). Some samples of the mineral are effective catalysts, promoting the formation of oligomers even from dilute solutions of activated nucleotide substrates. Furthermore, the mineral profoundly affects the regiospecificity of the reaction. The oligomerization of adenosine 5′-phosphorimidazolide, for example, gives predominantly 3′,5′-linked products in the presence of montmorillonite, but predominantly 2′,5′-linked products in aqueous solution (Ding et al. 1996; Kawamura and Ferris 1999). Once short oligomers have been synthesized, they can be further extended by adsorbing them on either montmorillonite or hydroxylapatite and repeatedly adding activated monomers, resulting in the accumulation of mainly 3′,5′-linked oligoadenylates up to 40–50 subunits in length (Ferris et al. 1996; Ferris 2002). However, even when adsorbed on montmorillonite, the phosphorimidazolides of the pyrimidine nucleosides yield oligomers that are predominantly 2′,5′-linked.

Long oligomers have also been obtained from monomers in a single step using a different activated nucleotide in which imidazole is replaced by 1-methyl-adenine (Prabahar and Ferris 1997; Huang and Ferris 2003). Using the 1-methyl-adenine derivative of adenylate or uridylate, oligomers containing up to 40 subunits were produced, consisting of ∼75% 3′,5′-linkages for oligoadenylate and ∼60% 3′,5′-linkages for oligouridylate (Huang and Ferris 2006). Oligomerization of the 1-methyl-adenine derivative of guanylate or cytidylate was less efficient, but all four activated monomers could be co-incorporated, to at least a modest extent, within abiotically synthesized oligonucleotides.

Detailed analysis of this work on catalysis by montmorillonite suggests that oligomerization occurs at a limited number of structurally specific active sites within the interlayers of the clay (Wang and Ferris 2001). These sites must not be saturated with sodium ions, which appear to block access of the activated nucleotides (Joshi et al. 2009). Several different samples of montmorillonite have proven to be good catalysts, in part depending on their proton versus sodium ion content. It will be important to determine if there are other types of minerals that are comparably efficient catalysts of oligonucleotide synthesis, and if so, to study the regiospecificity and sequence generality of the reactions they catalyze.

2.2 Nonenzymatic Replication of RNA

If a mechanism existed on the primitive Earth for the polymerization of activated nucleotides, it would have generated a complex mixture of product oligonucleotides that differed in both length and sequence. The next stage in the emergence of an RNA World would have been the replication of some of these molecules, so that a process equivalent to natural selection could begin. The reaction central to replication of nucleic acids is template-directed synthesis, that is, the synthesis of a complementary oligonucleotide under the direction of a preexisting oligonucleotide. A good deal of work has already been performed on this aspect of nonenzymatic replication. This work has been reviewed elsewhere (Joyce 1987; Orgel 2004a), so only a summary of the results will be given here.

The first major conclusion is that most activated nucleotides do not undergo efficient, regiospecific, template-directed reactions in the presence of an RNA or DNA template. In general, only a small proportion of template molecules succeed in directing the synthesis of a complete complement, and the complement usually contains a mixture of 2′,5′- and 3′,5′-phosphodiester linkages. After a considerable search, a set of activated nucleotides was found that undergo efficient and highly regiospecific template-directed reactions. Working with guanosine 5′-phospho-2-methylimidazolide (2-MeImpG), it was shown that poly(C) can direct the synthesis of long oligo(G)s in a reaction that is highly efficient and highly regiospecific (Inoue and Orgel 1981). If poly(C) is incubated with an equimolar mixture of the four 2-MeImp$N$s (N = G, A, C, or U), less than 1% of the product consists of noncomplementary nucleotides (Inoue and Orgel 1982). Subsequent experiments suggested that this and the related reactions discussed later occur preferentially within the context of double helices that have a structure resembling the A form of RNA (Kurz et al. 1997, 1998; Kozlov et al. 1999, 2000).

Random copolymers containing an excess of C residues can be used to direct the synthesis of products containing G and the complements of the other bases present in the template (Inoue and Orgel 1983). The reaction with a poly(C,G) template is especially interesting because the products, like the template, are composed entirely of C and G residues. If these products in turn could be used as templates, it might allow the emergence of a self-replicating sequence. Self-replication, however, is unlikely, mainly because poly(C,G) molecules that do not contain an excess of C residues tend to form stable self-structures that prevent them from acting as templates (Joyce and Orgel 1986). The self-structures are of two types: (1) the standard Watson-Crick variety based on C•G pairs, and (2) a quadrahelix structure that results from the association of four

G-rich sequences. As a consequence, any C-rich oligonucleotide that can serve as a good template will give rise to G-rich complementary products that tend to be locked in self-structure and so cannot act as templates. Overcoming the self-structure problem using the standard C and G nucleotides is very difficult because it requires the discovery of conditions that favor the binding of mononucleotides to allow template-directed synthesis to occur, but suppress the formation of long duplex regions that would exclude activated monomers from the template.

Some progress has been made in discovering defined-sequence templates that are copied faithfully to yield complementary products (Inoue et al. 1984; Acevedo and Orgel 1987; Wu and Orgel 1992a). Successful templates typically contain an excess of C residues, with A and U residues isolated from each other by at least three C residues. Runs of G residues are copied into runs of C residues, so long as the formation of self-structures by G residues can be avoided (Wu and Orgel 1992b). In light of the available evidence, it seems unlikely that a pair of complementary sequences can be found, each of which facilitates the synthesis of the other using nucleoside 5′-phospho-2-methylimidazolides as substrates. Some of the obstacles to self-replication may be attributable to the choice of reagents and reaction conditions, but others seem to be intrinsic to the template-directed condensation of activated mononucleotides.

A related nonenzymatic replication scheme involves synthesis by the ligation of short 3′,5′-linked oligomers (James and Ellington 1999). This is certainly an attractive possibility, made more plausible by the discovery of analogous ribozyme-catalyzed reactions (Bartel and Szostak 1993), but it faces two major obstacles. The first is the difficulty of obtaining the substrates in the first place. The second is concerned with fidelity. Pairs of oligonucleotides containing a single base mismatch, particularly if the mismatch forms a G•U wobble pair, still hybridize as efficiently as fully complementary oligomers, except in a temperature range very close to the melting point of the perfectly paired structure. Maintaining fidelity would therefore be difficult under any plausible temperature regime.

Despite these problems, template-directed ligation of short oligonucleotides may be a viable alternative to the oligomerization of activated monomers. Ferris' work discussed above suggests that predominantly 3′,5′-linked oligonucleotides might form spontaneously from activated nucleotides on some variety of montmorillonite (Ferris et al. 1996) or on some other mineral. Oligonucleotide 5′-triphosphates undergo slow but remarkably 3′,5′-regiospecific ligation in the presence of a complementary template (Rohatgi et al. 1996a,b). The combination of some such pair of reactions might provide a replication scheme for polynucleotides starting with an input of activated monomers.

There also are efforts in what is sometimes termed "synthetic biology" to achieve nonenzymatic replication with molecules that resemble biological nucleic acids, but are not constrained by considerations of plausible prebiotic chemistry. For example, the 2′- and 3′-hydroxyl groups of activated mononucleotides can be replaced by an amino group at either position, providing enhanced nucleophilicity and resulting in more rapid template-dependent (and template-independent) oligomerization (Lohrmann and Orgel 1976; Zielinski and Orgel 1985). Dinucleotide building blocks, consisting of 3′-amino, 3′-deoxynucleotide analogs can also be oligomerized in the presence of a suitable condensing agent (Zielinski and Orgel 1987). With additional modification of the nucleotide bases, it has been possible to carry out the template-directed copying of nucleic acid sequences that contain of all four bases (Schrum et al. 2009). These efforts, although not explaining the origin of the RNA World, contribute to understanding the chemical challenges that must be overcome in achieving the nonenzymatic replication of RNA.

2.3 The First RNA Replicase

The notion of the RNA World places emphasis on an RNA molecule that catalyzes its own replication. Such a molecule must function as an RNA-dependent RNA polymerase, acting on itself (or copies of itself) to produce complementary RNAs, and acting on the complementary RNAs to produce additional copies of itself. The efficiency and fidelity of this process must be sufficient to produce viable "progeny" RNA molecules at a rate that exceeds the rate of decomposition of the "parents." Beyond these requirements, the details of the replication process are not highly constrained.

The RNA-first view of the origin of life assumes that a supply of activated ß-D-nucleotides was available by some as yet unrecognized abiotic process. Furthermore, it assumes that a means existed to convert the activated nucleotides to an ensemble of random-sequence polynucleotides, a subset of which had the ability to replicate. It seems to be implicit in the model that such polynucleotides replicate themselves but, for whatever reason, do not replicate unrelated neighbors. It is not clear whether replication involves one molecule copying itself (and its complement) or a family of molecules that together copy each other. These questions are set aside for the moment in order to first consider the question of whether an RNA molecule of reasonably short length can catalyze its own replication with sufficiently high fidelity.

Accuracy and Survival. The concept of an error threshold, that is, an upper limit to the frequency of copying errors that can be tolerated by a replicating macromolecule, was first introduced by Eigen (1971). This important idea has been extended in a series of mathematically sophisticated papers by McCaskill, Schuster, and others (McCaskill 1984a; Eigen et al. 1988; Schuster and Swetina 1988). Here only a brief summary of the subject is provided.

Eigen's model (1971) envisages a population of replicating polynucleotides that draw on a limited supply of activated mononucleotides to produce additional copies of themselves. In this model, the rate of synthesis of new copies of a particular replicating RNA is proportional to its concentration, resulting in autocatalytic growth. The net rate of production is the difference between the rate of formation of error-free copies and the rate of decomposition of existing copies of the RNA. For an advantageous RNA to outgrow its competitors, its net rate of production must exceed the mean rate of production of all other RNAs in the population. Only the error-free copies of the advantageous RNA contribute to its net rate of production, but all the copies of the other RNAs contribute to their collective production. Thus the relative advantage enjoyed by the advantageous individual compared with the rest of the population (often referred to as the "superiority" of the advantageous individual) must exceed the probability of producing an error copy of that advantageous individual.

The proportion of copies of an RNA that are error free is determined by the fidelity of the component condensation reactions that are required to produce a complete copy. For simplicity, consider a self-replicating RNA that is formed by n condensation reactions, each having mean fidelity q. The probability of obtaining a completely error-free copy is given by q^n, which is the product of the fidelity of the component condensation reactions. If an advantageous individual is to outgrow its competitors, q^n must exceed the superiority, s, of that individual. Expressed in terms of the number of reactions required to produce the advantageous individual,

$$n < |\ln s| / |\ln q|.$$

For $s > 1$ and $q > 0.9$, this equation simplifies to

$$n < \ln s / (1 - q).$$

This is the "error threshold," which describes the inverse relationship between the fidelity of replication, q, and the maximum allowable number of component condensation reactions, n. The maximum number of component reactions is highly sensitive to the fidelity of replication, but depends only weakly on the superiority of the advantageous individual. For a self-replicating RNA that is formed by the template-directed condensation of activated mononucleotides, a total of $2n - 2$ condensation reactions are required to produce a complete copy. This takes into account the synthesis of both a complementary strand and a complement of the complement.

It should be recognized that a marked superiority of one sequence over all other sequences could not be maintained over evolutionary time because novel variants would soon arise to challenge the dominant species. However, a marked initial superiority may be important in allowing an efficient self-replicating RNA to emerge from a pool of less efficient replicators. In the absence of other efficient replicators, a primitive self-replicating RNA that operates with low fidelity may gain a foothold by taking advantage of a somewhat less stringent error threshold. Whether or not this can occur depends on its superiority. For example, an RNA that replicates 10-fold more efficiently than its competitors and does so with 90% fidelity could be no longer than 12 nucleotides, and a similarly advantageous RNA that replicates with 70% fidelity could be no longer than four nucleotides. It seems highly unlikely than *any* of the 17 million possible RNA dodecamers is able to catalyze its own replication with 90% fidelity, and even less likely that a tetranucleotide could catalyze its own replication with 70% fidelity. However, an RNA that replicates 10^6-fold more efficiently than its competitors and does so with 90% fidelity could be as long as 67 nucleotides, and one that replicates with 70% fidelity could be as long as 20 nucleotides.

When self-replication is first established, fidelity is likely to be poor and there is strong selection pressure favoring improvement of the fidelity. As fidelity improves, a larger genome can be maintained. This allows exploration of a larger number of possible sequences, some of which may lead to further improvement in fidelity, which in turn allows a still larger genome size, and so on. Once the evolving population has achieved a fidelity of about 99%, a genome length of about 100 nucleotides can be maintained, even for modest superiority values. This would allow RNA-based life to become firmly established. Until that time, it is a race between evolutionary improvement in the context of a sloppy self-replicating system and the risk of delocalization of the genetic information because of overstepping the error threshold. If the time required to bootstrap to high fidelity and large genomes is too long, there is a risk that the population will succumb to an environmental catastrophe before it has had the chance to develop appropriate countermeasures.

It is difficult to state with certainty the minimum possible size of an RNA replicase ribozyme. An RNA consisting

of a single secondary structural element, that is, a small stem-loop containing 12–17 nucleotides, would not be expected to have replicase activity, whereas a double stem-loop, perhaps forming a "dumbbell" structure or a pseudoknot, might just be capable of a low level of activity. A triple stem-loop structure, containing 40–60 nucleotides, offers a reasonable hope of functioning as a replicase ribozyme. One could, for example, imagine a molecule consisting of a pseudoknot and a pendant stem-loop that forms a cleft for template-dependent replication.

Suppose there is some 40-mer that enjoys a superiority of 10^3-fold and replicates with 90% fidelity. This should be regarded as a highly optimistic but not outrageous view of what is possible for a minimum replicase ribozyme. Would such a molecule be expected to occur within a population of random-sequence RNAs? A complete library consisting of one copy each of all 10^{24} possible 40-mers would weigh about 1 kg. There may be many such 40-mers, encompassing both distinct structural motifs and, more importantly, a large number of equivalent representations of each motif. As a result, even a small fraction of the total library, consisting of perhaps 10^{20} sequences and weighing about 1 g, might be expected to contain at least one self-replicating RNA with the requisite properties. It is not sufficient, however, that there be just one copy of a self-replicating RNA. The above calculations assume that a self-replicating RNA can copy itself (or that a fully complementary sequence is automatically available, as will be discussed later). If two or more copies of the same 40mer RNA are needed, then a much larger library, consisting of 10^{48} RNAs and weighing 10^{28} g would be required. This amount is comparable to the mass of the Earth.

At first sight, it might seem that one way to ease the error threshold would be for the replicase ribozyme to accept dinucleotide or trinucleotide substrates, so that copies of the RNA could be formed by fewer condensation reactions. Calculations show that, over a broad range of superiority values, RNAs that are required to replicate with 90% fidelity when using mononucleotide substrates would be required to replicate with roughly 80% fidelity when using dinucleotide substrates or roughly 70% fidelity when using trinucleotide substrates. Thus the use of short oligomers offers only a modest advantage because of lessening of the error threshold, which likely would be outweighed by the greater difficulty of achieving high fidelity when discriminating among the 16 possible dinucleotide or 64 possible trinucleotide substrates, rather than among the four mononucleotides.

If one accepts the RNA-first view that there was a prebiotic pool of random-sequence RNAs, and if one assumes that the pool included a replicase ribozyme containing, say, 40 nucleotides and replicating itself with about 90%

fidelity, then it is not difficult to imagine how RNA-based evolution might have started. During the initial period a successful clone would have expanded in the absence of competition. As competition for substrates intensified there would have followed a succession of increasingly more advantageous individuals, each replicating within its error threshold. After a period of intensifying competition, the single most advantageous species would have been replaced by a "quasispecies," that is, a mixture of the most advantageous individual and substantial amounts of closely related individuals that replicate almost as fast and almost as faithfully as the most advantageous one (Eigen and Schuster 1977; Eigen et al. 1988). Under these conditions the persistence of a particular advantageous individual is no longer the problem, but one must understand the evolution of the composition of the quasispecies and the conditions for its persistence. This difficult problem has been partially solved by McCaskill (1984b). The general form of the solution is very similar to the error threshold described by Eigen (1971), but with different values for the constant in the inequality. Thus concerns about the error threshold apply to the quasispecies as well as to the succession of individuals. Practically speaking, however, once a quasispecies distribution of sophisticated replicators had emerged, the RNA World would have been on solid footing and, barring an environmental catastrophe, unlikely to lose the ability to maintain genetic information over time.

Another Chicken-and-Egg Paradox. The previous discussion has tried mightily to present the most optimistic view possible for the emergence of an RNA replicase ribozyme from a soup of random-sequence polynucleotides. It must be admitted, however, that this model does not appear to be very plausible. The discussion has focused on a straw man: The myth of a small RNA molecule that arises de novo and can replicate efficiently and with high fidelity under plausible prebiotic conditions. Not only is such a notion unrealistic in light of current understanding of prebiotic chemistry (Joyce 2002), but it should strain the credulity of even an optimist's view of RNA's catalytic potential. If you doubt this, ask yourself whether you believe that a replicase ribozyme would arise in a solution containing nucleoside 5′-diphosphates and polynucleotide phosphorylase!

If one accepts the notion of an RNA World, one is faced with the dilemma of how such a genetic system came into existence. To say that the RNA World hypothesis "solves the paradox of the chicken-and-the-egg" is correct if one means that RNA can function both as a genetic molecule and as a catalyst that promotes its own replication. RNA-catalyzed RNA replication provides a chemical basis for

Darwinian evolution based on natural selection. Darwinian evolution is a powerful way to search among vast numbers of potential solutions for those that best address a particular problem. Selection based on inefficient RNA replication, for example, could be used to search among a population of RNA molecules for those individuals that promote improved RNA replication. But here one encounters another chicken-and-egg paradox: Without evolution it appears unlikely that a self-replicating ribozyme could arise, but without some form of self-replication there is no way to conduct an evolutionary search for the first, primitive self-replicating ribozyme.

One way that RNA evolution may have gotten started without the aid of an evolved catalyst might be by using nonenzymatic template-directed synthesis to permit some copying of RNA before the appearance of the first replicase ribozyme. Suppose that the initial ensemble of monomers was not produced by random copolymerization, but rather by a sequence of untemplated and templated reactions (Fig. 2), and further suppose that members of the initial ensemble of multiple stem-loop structures could be replicated, albeit inefficiently, by the template-directed process. This would have two important consequences. First, any molecule with replicase function that appeared in the mixture would likely find in its neighborhood similar

molecules and their complements, related by descent, thus eliminating the requirement for two unrelated replicases to meet. Second, a majority of molecules in the mixture would contain stem-loop structures. If it is true that ribozyme function is favored by stable self-structure, and if the base-sequences of the stems in stem-loop structures are relatively unimportant for function, this model might provide an economical way of generating a relatively small ensemble of sequences that is enriched with catalytic sequences.

How plausible is the assumption that replicases could act on sequences similar to themselves, while ignoring unrelated sequences? This selectivity could be ensured by segregating individual molecules (or clonal lines) on the surface of mineral grains, on the surface of micelles, or within membranes. Closely related molecules might be segregated as a group through specific hydrogen-bonding interactions (the family that sticks together, replicates together). For any segregation mechanism, weak selection would result if the replicating molecules are sufficiently dispersed that diffusion over their intermolecular distance is slow compared with replication. Computer simulations have shown that under such conditions of segregation, evolutionary bootstrapping can occur, resulting in progressively larger genomes that are copied with progressively greater fidelity (Szabó et al. 2002). Alternatively, the
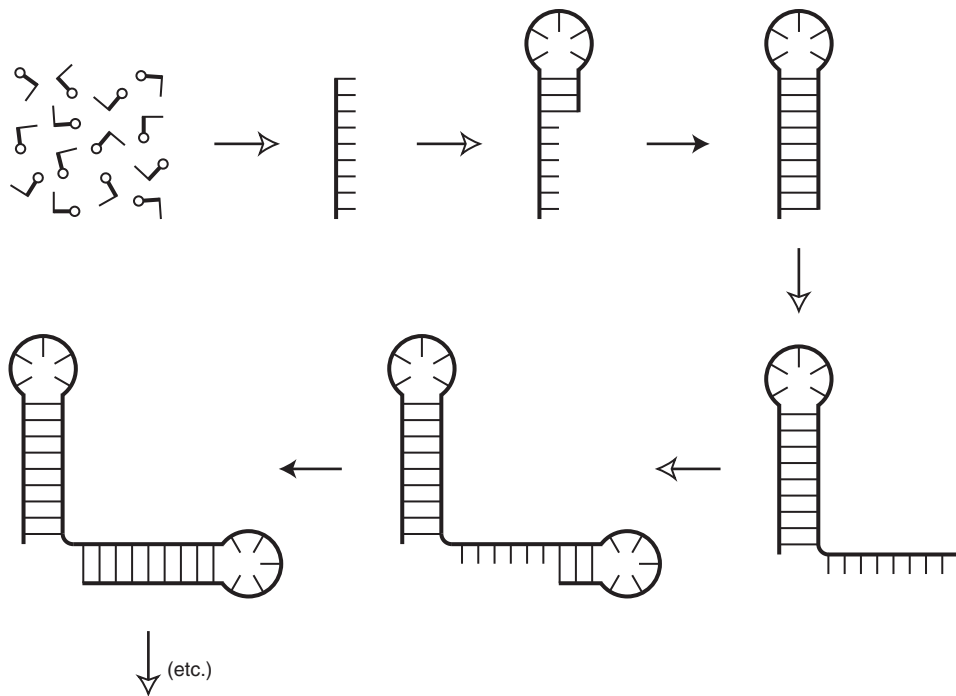
Figure 2. Nonenzymatic synthesis of multi-stem-loop structures as a result of untemplated (open arrowhead) and templated (filled arrowhead) reactions. Template-directed synthesis is assumed to occur rapidly whenever a template, activated monomers, and a suitable primer are available. Once the complementary strand is completed, additional residues are added slowly in a random-sequence manner.

requirement for replication of related, but not unrelated, sequences might be met through the use of "genomic tags" (Weiner and Maizels 1987). Among self-replicating sequences, it is plausible that some are restricted to copying molecules with a particular 3′-terminal subsequence. A replicator that happened by chance to carry a terminal sequence that matched the preference of its active site would replicate itself while ignoring its neighbors.

Another resolution of the paradox of how RNA evolution was initiated without the aid of an evolved ribozyme is to abandon the RNA-first view of the origin of life and suppose that RNA was *not* the first genetic molecule (Cairns-Smith 1982; Shapiro 1984; Joyce et al. 1987; Joyce 1989, 2002; Orgel 1989, 2004a). Perhaps RNA replication arose in the context of an evolving system based on something other than RNA (see the section "Alternative Genetic Systems"). Even if this is true, all of the arguments concerning the relationship between the fidelity of replication and the maximum allowable genome length would still apply to this earlier genetic system. Of course, the challenge to those who advocate the RNA-later approach is to show that there is an informational entity that is prebiotically plausible and is capable of initiating its own replication without the aid of a sophisticated catalyst.

2.4 Replicase Function in the Evolved RNA World

Although it is difficult to say how the first RNA replicase ribozyme arose, it is not difficult to imagine how such a molecule, once developed, would function. The chemistry of RNA replication would involve the template-directed polymerization of mono- or short oligonucleotides, using chemistry in many ways similar to that used by contemporary group I ribozymes (Cech 1986; Been and Cech 1988; Doudna and Szostak 1989). One important difference is that, unlike group I ribozymes, which rely on a nucleoside or oligonucleotide leaving group, an RNA replicase would more likely make use of a different leaving group that provides a substantial driving force for polymerization and that, after its release, does not become involved in some competing phosphoester transfer reaction.

From Ligases to Polymerases. The polymerization of activated nucleotides proceeds via nucleophilic attack by the 3′-hydroxyl of a template-bound oligonucleotide at the α-phosphorus of an adjacent template-bound nucleotide derivative (Fig. 3). The nucleotide is "activated" for attack by the presence of a phosphoryl substituent, for example a phosphate, polyphosphate, alkoxide, or imidazole group. As discussed previously, polyphosphates, such as inorganic pyrophosphate, are the most obvious candidates for the leaving group. The condensation reaction could be assisted
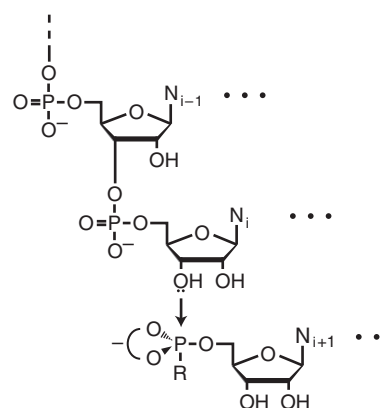

Figure 3. Nucleophilic attack by the 3′-hydroxyl of a template-bound oligonucleotide (N_1-N_i) on the α-phosphorus of an adjacent template-bound mononucleotide (N_{i+1}). Dotted lines indicate base pairing to a complementary template. R is the leaving group.

by favorable orientation of the reactive groups, deprotonation of the nucleophilic 3′-hydroxyl, stabilization of the trigonal-bipyramidal transition state, and charge neutralization of the leaving group. All of these tasks might be performed by RNA (Narlikar and Herschlag 1997; Emilsson et al. 2003), acting either alone (Ortoleva-Donnelly and Strobel 1999) or with the help of a suitably positioned metal cation or other cofactor (Shan et al. 1999; Shan et al. 2001).

The possibility that an RNA replicase ribozyme could have existed has been made abundantly clear by work involving ribozymes that have been developed in the laboratory through in vitro evolution (Bartel and Szostak 1993; Ekland et al. 1995; Ekland and Bartel 1996; Robertson and Ellington 1999; Jaeger et al. 1999; Rogers and Joyce 2001; Johnston et al. 2001; McGinness and Joyce 2002; Ikawa et al. 2004; Fujita et al. 2009). Bartel and Szostak (1993), for example, began with a large population of random-sequence RNAs and evolved the "class I" RNA ligase ribozyme, an optimized version of which is about 100 nucleotides in length and catalyzes the joining of two template-bound oligonucleotides. Condensation occurs between the 3′-hydroxyl of one oligonucleotide and the 5′-triphosphate of another, forming a 3′,5′-phosphodiester linkage and releasing inorganic pyrophosphate. This reaction is classified as ligation because of the nature of the oligonucleotide substrates, but involves the same chemical transformation as is catalyzed by modern RNA polymerase enzymes.

X-ray crystal structures of two RNA ligase ribozymes, the L1 and above-mentioned class I ligases, have been determined, providing a glimpse into the mechanistic strategies that these two structurally and evolutionarily distinct ribozymes use to catalyze the same reaction (Robertson and
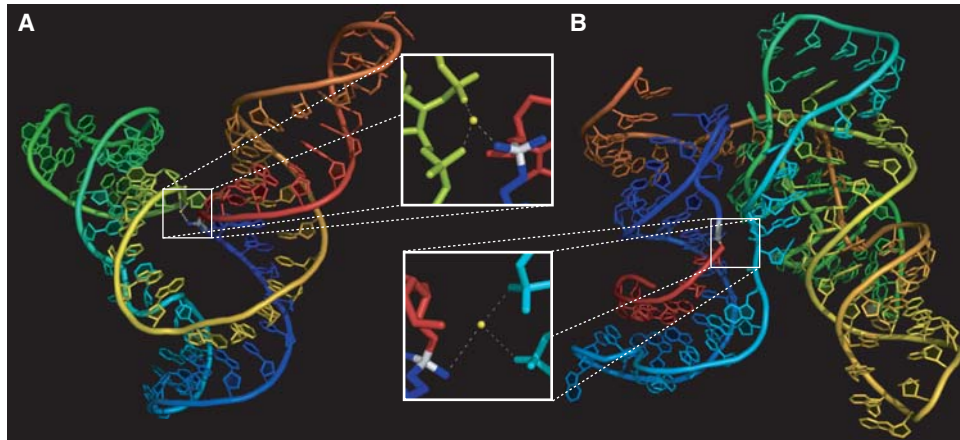
Figure 4. X-ray crystal structure of the (*A*) L1 ligase and (*B*) class I ligase ribozymes. Insets show the putative magnesium ion binding sites at the respective ligation junctions. The structures are rendered in rainbow continuum, with the 5′-triphosphate-bearing end of the ribozyme colored violet and the 3′-hydroxyl-bearing end of the substrate colored red. The phosphate at the ligation junction is shown in white, and the proximate magnesium ion (modeled for the class I ligase) is shown as a yellow sphere, with dashed lines indicating coordination contacts.

Scott 2007; Shechner et al. 2009) (Fig. 4). Both crystal structures capture the product of the ligation reaction, and consequently offer an incomplete view of the reaction pathway. For example, the pyrophosphate leaving group is absent from the structures, so no conclusions can be drawn regarding potential ribozyme-assisted orientation of the reactive triphosphate or charge neutralization of the pyrophosphate leaving group. There is, however, information regarding other aspects of the reaction mechanism that can be inferred from the product structures.

Both the L1 and class I ligases are dependent on the presence of magnesium ions for their activity. A prominent feature of the L1 structure (Fig. 4A) involves a bound metal ion in the active site, coordinated by three nonbridging phosphate oxygens, one of which belongs to the newly formed phosphodiester linking what originally were the two substrates. This magnesium ion is favorably positioned to help neutralize the increased negative charge of the transition state and, potentially, to activate the 3′-hydroxyl nucleophile and to help orient the α-phosphate for a more optimal in-line alignment. In the case of the class I structure (Fig. 4B), no catalytic metal ions appear to have been retained in the vicinity of the active site, although two magnesium ions are observed to participate in crucial structural interactions that help shape the active site architecture. Despite the lack of direct observation of a catalytic metal at the active site, there is what appears to be an empty metal binding site formed by two nonbridging phosphate oxygens, positioned directly opposite the ligation junction in a manner similar to that observed for the magnesium binding site of the L1 ligase and reminiscent of the arrangement seen in protein polymerases. The lack of a metal in the

crystal structure may simply be an artifact of the crystallization process or may imply a local conformational change in the product that disfavors retention of the bound metal. These structures show that, despite some remaining gaps in the detailed understanding of how these ribozymes function, the available information points to a universal catalytic strategy, very similar to that used by modern protein-based RNA polymerases.

Subsequent to its isolation as a ligase, the class I ribozyme was shown to catalyze a polymerization reaction in which the 5′-triphosphate-bearing oligonucleotide is replaced by one or more NTPs (Ekland and Bartel 1996). This reaction proceeds with high fidelity ($q = 0.92$), but the reaction rate drops sharply with successive nucleotide additions.

Bartel and colleagues performed further in vitro evolution experiments to convert the class I ligase to a bona fide RNA polymerase that operates on a separate RNA template (Johnston et al. 2001). To the 3′ end of the class I ligase they added 76 random-sequence nucleotides that were evolved to form an accessory domain that assists in the polymerization of template-bound NTPs. The polymerization reaction is applicable to a variety of template sequences, and for well-behaved sequences proceeds with an average fidelity of 0.967. This would be sufficient to support a genome length of about 30 nucleotides, although the ribozyme itself contains about 190 nucleotides. The ribozyme has a catalytic rate for NTP addition of at least 1.5 min^{-1}, but its K_m is so high that, even in the presence of micromolar concentrations of oligonucleotides and millimolar concentrations of NTPs, it requires about 2 h to complete each NTP addition (Lawrence and Bartel 2003). The ribozyme

operates best under conditions of high Mg^{2+} concentration, but becomes degraded under those conditions over 24 h, by which time it has added no more than 14 NTPs (Johnston et al. 2001).

Further optimization of the polymerase ribozyme using highly sophisticated in vitro evolution techniques has led to additional improvements in its biochemical properties. By directly selecting for extension of an external primer on a separate template, Zaher and Unrau (2007) were able to improve the maximum length of template-dependent polymerization to >20 nucleotides, with a rate that is ~threefold faster than that of the parent for the first nine monomer additions and up to 75-fold faster for additions beyond 10 nucleotides. In addition, although not rigorously quantitated, the new ribozyme displays significantly improved fidelity, particularly with respect to discrimination against G•U wobble pairs. It is this improved fidelity that appears to be the underlying source for the observed improvements in the maximum length of extension and the rate of polymerization.

A different RNA ligase ribozyme can operate on a separate RNA template in a largely sequence-general manner, and does so with a K_m that is at least 100-fold lower than that of the class-I-derived polymerase (McGinness and Joyce 2002). However, its catalytic rate is much lower as well, and it is unable to add more than a single NTP. Yet another RNA ligase ribozyme can operate on a separate template with the help of designed tertiary interactions that "clamp" the template–substrate complex to the ribozyme (Ikawa et al. 2004). But it too is a relatively slow catalyst that cannot add more than a single NTP.

A highly pessimistic view is that because there is no known polymerase ribozyme that combines all of the properties necessary to sustain its own replication, no such ribozyme is possible. A more balanced view is that RNA clearly is capable of greatly accelerating the template-dependent polymerization of nucleoside 5′-polyphosphates. Such catalytic RNAs can operate in a sequence-general manner and with reasonable fidelity. It seems only a matter of time (and likely considerable effort) before more robust polymerase ribozymes will be obtained. Nature did not have the opportunity to conduct carefully arranged evolution experiments using highly-purified reagents, but did have the luxury of much greater reaction volumes and much more time.

RNA Replication. Despite falling short of the ultimate goal of a general-purpose RNA polymerase ribozyme, a robust reaction system for RNA-catalyzed RNA replication has recently been shown. The system uses a pair of cross-replicating ligase ribozymes that each catalyze the formation of the other, using a mixture of four different substrate oligonucleotides (Lincoln and Joyce 2009). In reaction mixtures containing only these RNA substrates, $MgCl_2$, and buffer, a small starting amount of ribozymes gives rise to many additional ribozymes through a process of RNA-catalyzed exponential amplification. Whenever the substrates become depleted, the replication process can be restarted and sustained indefinitely by replenishing the supply of substrates.

Because the substrates are recognized by the ribozymes through specific Watson-Crick pairing interactions, evolution experiments can be performed by providing a variety of substrates that have different sequences in these recognition regions and different corresponding sequences in the catalytic domain of the ribozyme. RNA replication was performed with a library of 144 possible substrate combinations, resulting in the emergence of a set of highly advantageous replicators that included recombinants which were not present at the start of the experiment. Until the advent of a general-purpose RNA polymerase ribozyme, the system of cross-replicating ligases offers the best platform to study the biochemical properties and evolutionary behavior of an all-RNA replicative system.

Nucleotide Biosynthesis. RNA replicase activity is probably not the only catalytic behavior that was essential for the existence of the RNA World. Maintaining an adequate supply of the four activated nucleotides would have been a top priority. Even if the prebiotic environment contained a large reservoir of these compounds, the reservoir would eventually become depleted, and some capacity for nucleotide biosynthesis would have been required.

Ribozymes have been obtained, through in vitro evolution, that catalyze some of the steps of nucleotide biosynthesis. Unrau and Bartel (1998), for example, developed a ribozyme that catalyzes a reaction between 4-thiouracil and 5-phosphoribosyl-1-pyrophosphate (PRPP) to form 4-thiouridylate (Fig. 5A). The 4-thiouracil is provided free in solution and the PRPP is tethered to the 3′ end of the ribozyme. An optimized form of this ribozyme, containing 124 nucleotides, has an observed rate of 0.2 min^{-1} in the presence of 4 mM 4-thiouracil (Chapple et al. 2003). This is at least 10^7-fold faster than the uncatalyzed rate of reaction, which is too slow to measure. Unrau and colleagues used a similar approach to develop two different ribozymes that catalyze the formation of 6-thioguanylate from 6-thioguanine and tethered PRPP (Lau et al. 2004), as well as a third guanylate synthase ribozyme that arose as an unanticipated consequence of a related in vitro evolution experiment (Lau and Unrau 2009). The first two guanylate synthase ribozymes are slightly larger and have about twofold higher catalytic efficiency compared
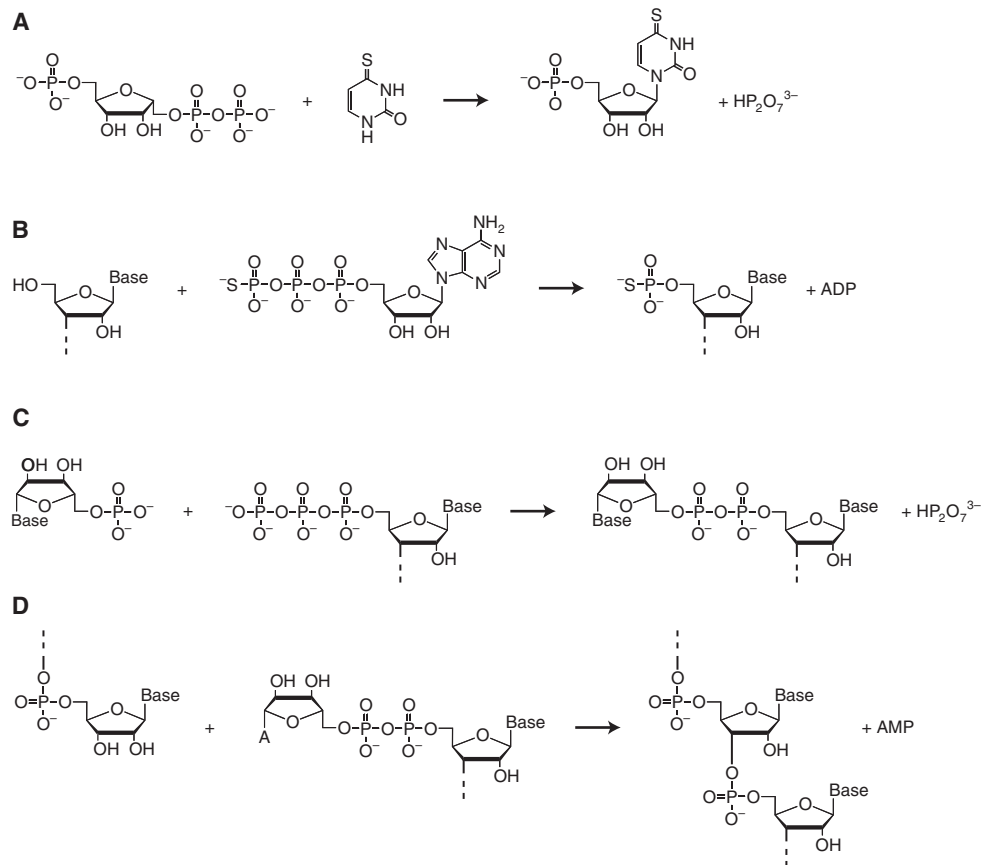
Figure 5. Known RNA-catalyzed reactions that are relevant to nucleotide biosynthesis. (*A*) Formation of 4-thiouridylate from free 4-thiouracil and ribozyme-tethered 5-phosphoribosyl-1-pyrophosphate. (*B*) 5′-phosphorylation of an oligonucleotide using γ-thio-ATP as the phosphate donor. (*C*) Activation of a nucleoside 5′-phosphate by formation of a 5′,5′-pyrophosphate linkage. (*D*) Template-directed ligation of RNA driven by release of a 5′,5′-pyrophosphate-linked adenylate.

with the uridylate synthase ribozyme, although guanylate synthesis is expected to have a much higher uncatalyzed rate of reaction.

RNA-catalyzed synthesis of PRPP has not been shown, but a ribozyme has been obtained that catalyzes the 5′-phosphorylation of oligonucleotides using γ-thio-ATP as the phosphate donor (Lorsch and Szostak 1994) (Fig. 5B). The ribozyme shows a rate enhancement of about 10^9-fold compared with the uncatalyzed rate of reaction. Once a nucleoside 5′-phosphate has been formed, it can be activated by another ribozyme that catalyzes the condensation of a nucleoside 5′-phosphate and a ribozyme-tethered nucleoside 5′-triphosphate (Huang and Yarus 1997) (Fig. 5C). This results in the formation of a 5′,5′-pyrophosphate linkage, which provides an activated nucleotide leaving group that can drive subsequent RNA-catalyzed, template-directed ligation of RNA (Hager and Szostak 1997) (Fig. 5D).

None of these four RNA-catalyzed reactions has precisely the right format for the corresponding reaction in a hypothetical nucleotide biosynthesis pathway in the RNA World. However, they show that RNA is capable of performing the relevant chemistry with substantial catalytic rate enhancement. It remains to be seen whether ribozymes can be developed that catalyze the formation of the fundamental building blocks of RNA, D-ribose and the four nucleotide bases, using starting materials that would have been abundant on the primitive Earth.

3 AN "RNA-LATER" VIEW OF THE ORIGIN OF LIFE

3.1 Abiotic Synthesis of Nucleotides

The RNA-first view of the origin of life proceeds from the assumption that pure ß-D-nucleotides were available in some prebiotic pool. How close to such a pool could one

hope to get without magic (or evolved enzymes) on the primitive Earth? Could one hope to achieve replication in a pool containing a more realistic mixture of organic molecules, including, of course, ß-ᴅ-ribonucleotides? The synthesis of a nucleotide could occur in a number of ways. The simplest, conceptually, would be to synthesize a nucleoside base, couple it to ribose, and finally to phosphorylate the resulting nucleoside. However, a number of other routes are feasible, for example the assembly of the base on a preformed ribose or ribose phosphate, or the coassembly of the base and sugar-phosphate.

The classical prebiotic synthesis of sugars is by the polymerization of formaldehyde (the "formose" reaction). It yields a very complex mixture of products including only a small proportion of ribose (Mizuno and Weiss 1974). This reaction does not provide a reasonable route to the ribonucleotides. However, a number of more recent experimental findings, to some extent, address this deficiency.

The base-catalyzed aldomerization of glycoaldehyde phosphate in the presence of a half-equivalent of formaldehyde under strongly alkaline conditions gives a relatively simple mixture of tetrose- and pentose-diphosphates and hexose-triphosphates, of which ribose 2,4-diphosphate is the major component (Müller et al. 1990). Reactions of this kind proceed efficiently when 2 mM solutions of substrates are incubated at room temperature and pH 9.5 in the presence of layered hydroxides such as hydrotalcite (magnesium aluminum hydroxide) (Pitsch 1992; Pitsch et al. 1995a). The phosphates are absorbed between the positively charged layers of the mineral. The reaction proceeds under these milder conditions presumably because of the high concentration of substrates in the interlayer and because the positive charge on the metal hydroxide layers favors enolization of glycoaldehyde phosphate. A reaction between glycoaldehyde or glyceraldehyde and the amidotriphosphate ion provides an ingenious and prebiotically plausible route to glycoaldehyde phosphate and glyceraldehyde-2-phosphate, respectively, the two substrates in the above reactions (Krishnamurthy et al. 2000).

A number of other studies have addressed the problems presented by the lack of specificity of the formose reaction and by the instability of ribose. The Pb^{2+} ion is an excellent catalyst for the formose reaction and enables yields of the pentose sugars as high as 30% to be achieved (Zubay 1998). Furthermore, it seems likely that ribose is almost exclusively the first pentose product of the reaction and that the other pentoses are formed from it by isomerization. Other recent studies have addressed the problem presented by the instability of ribose. The four pentose sugars, including ribose, are all strongly stabilized in the presence of borate ions or calcium borate minerals (Ricardo et al. 2004). However, the effect of borate on the progress of the formose reaction has not been reported.

Many sugars, including the four pentoses, react readily with cyanamide to form stable bicyclic amino-oxazolines (Sanchez and Orgel 1970) (Fig. 6A). Strikingly, the ribose derivative crystallizes readily from aqueous solution even when complex mixtures of related molecules, including a mixture of the amino-oxazoline derivatives of the other three pentose sugars, are present (Springsteen and Joyce 2004). The crystals are multiply twinned, each crystal containing many small domains of each of the two enantiomorphs. Thus the reaction of a mixture of racemic sugars with cyanamide followed by crystallization might stabilize ribose, segregate it from other sugars, and present it in enantiospecific microdomains. Much remains to be shown, but the reactions described above suggest that ribose synthesis, although still problematic, may not be the intractable problem it once seemed.

The synthesis of the nucleoside bases is one of the success stories of prebiotic chemistry. Adenine is formed with remarkable ease from ammonia and hydrogen cyanide (Orò 1961). This synthesis has been described as "the rock of the faith" by Stanley Miller. Reasonably plausible syntheses of the other purine bases and of the pyrimidines have also been described (Sanchez et al. 1967; Ferris et al. 1968; Robertson and Miller 1995; Orgel 2004b; Saladino et al. 2004). The coupling of the purine bases with ribose or ribose-phosphate has been achieved under mild conditions, but in relatively low yield (Fuller et al. 1972). The corresponding reaction with pyrimidines does not occur.

There is a different potential route for the prebiotic synthesis of pyrimidine nucleotides via arabinose amino-oxazoline that first was explored nearly 40 yr ago (Tapiero and Nagyvary 1971) and in recent years has begun to look very persuasive (Ingar et al. 2003; Anastasi et al. 2007; Powner et al. 2009). The earlier studies began with arabinose 3-phosphate, which, like arabinose and other sugars, reacts with cyanamide to give the corresponding amino-oxazoline (Fig. 6B). This in turn reacts with cyanoacetylene to form a tricyclic intermediate that hydrolyzes to produce a mixture of cytosine arabinoside-3′-phosphate and cytosine 2′,3′-cyclic phosphate.

Sutherland and colleagues (Powner et al. 2009) have taken this approach further by starting simply with glycoaldehyde and cyanamide, which in the presence of 1 M phosphate at neutral pH gives 2-amino-oxazole in excellent yield (Fig. 6C). The phosphate both buffers and catalyzes the reaction, directing glycoaldehyde toward 2-amino-oxazole, rather than a complex mixture of aldomerization products. Glyceraldehyde is then added, resulting in formation of the various pentose amino-oxazolines, including
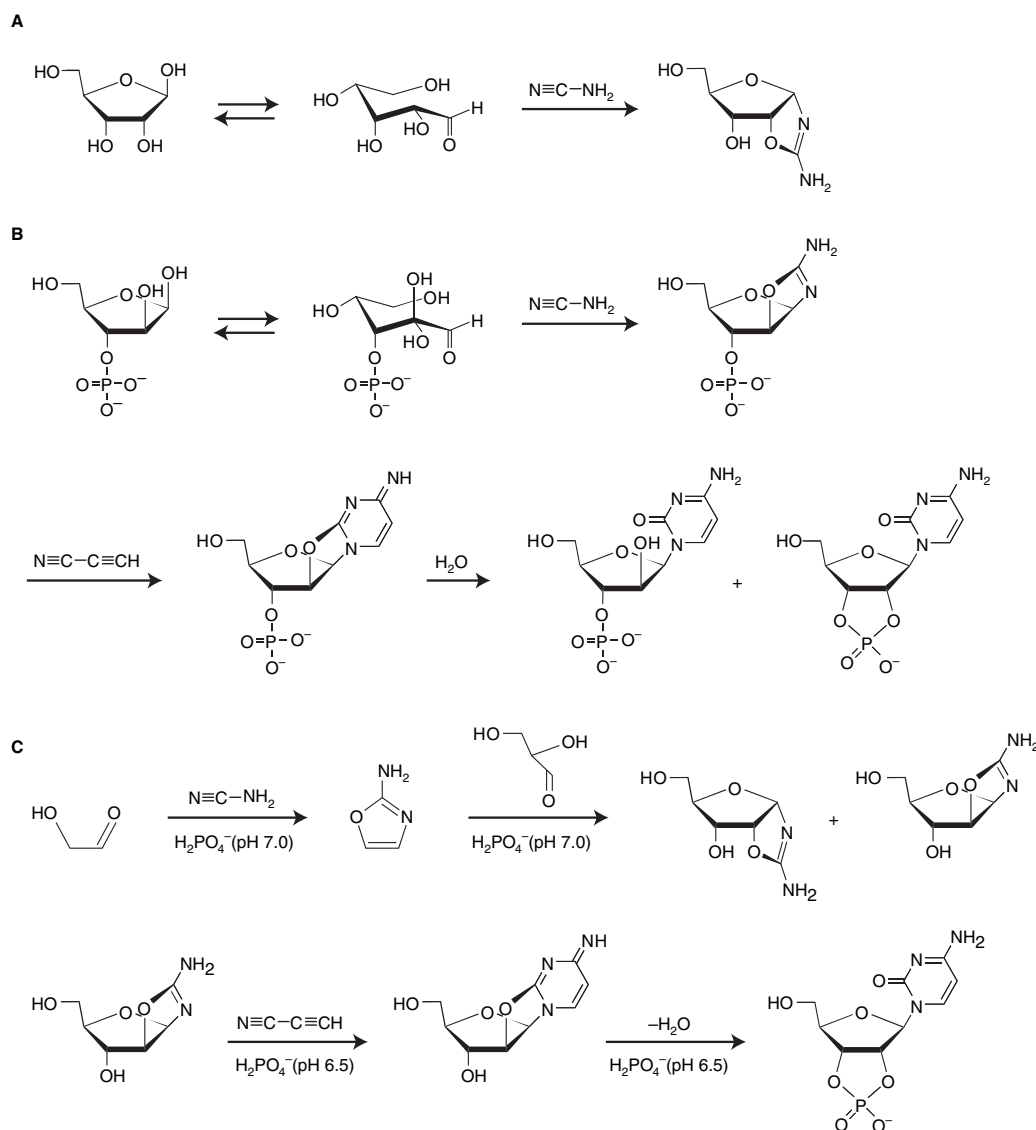
Figure 6. Potential prebiotic synthesis of pyrimidine nucleosides. (*A*) Reaction of ribose with cyanamide to form a bicyclic product, with cyanamide joined at both the anomeric carbon and 2-hydroxyl. (*B*) Analogous reaction of arabinose-3-phosphate to form a bicyclic product, which then reacts with cyanoacetylene to form a tricyclic intermediate that hydrolyzes to give a mixture of cytosine arabinoside-3′-phosphate and cytosine 2′,3′-cyclic phosphate. (*C*) Reaction of glycoaldehyde with cyanamide in neutral phosphate buffer, followed by addition of glyceraldehyde, to form ribose and arabinose amino-oxazoline (and lesser amounts of the xylose and lyxose compounds). Arabinose amino-oxazoline then reacts with cyanoacetylene to give cytosine 2′,3′-cyclic phosphate as the major product.

the arabinose compound. Arabinose amino-oxazoline, in turn, can react with cyanoacetylene, also in phosphate buffer, to form cytosine 2′,3′-cyclic phosphate as the major product. Perhaps equally intriguing, although given less emphasis in these studies, is that reaction of arabinose amino-oxazoline with cyanoacetylene also gives a substantial yield of cytosine 2′,3′-cyclic-5′-bisphosphate, which is more amenable to being converted to an activated monomer that would be suitable for polymerization.

There has been significant progress, especially recently, concerning the synthesis of the nucleosides and nucleotides from prebiotic precursors in reasonable yield. However, the story remains incomplete because these syntheses still require temporally separated reactions using high concentrations of just the right reactants, and would be disrupted by the presence of other closely related compounds. The reactions channel material toward the desired products, but other fractionation processes must be discovered that

provide the correct starting materials at the requisite time and place. This "preprebiotic" chemistry likely would involve a series of reactions catalyzed by minerals or metal ions, coupled with a series of subtle fractionations of nucleotide-like materials based on adsorption on minerals, selective complex formation, crystallization, etc.

Even minerals could not achieve on a macroscopic scale one desirable separation, the resolution of D-ribonucleotides from their L-enantiomers. This is a serious problem because experiments on template-directed synthesis using poly(C) and the imidazolides of G suggest that the polymerization of the D-enantiomer is strongly inhibited by the L-enantiomer (Joyce et al. 1984). This difficulty may not be insuperable; perhaps with a different mode of phosphate activation, the inhibition would be less severe. However, enantiomeric cross-inhibition is certainly a serious problem if life arose in a racemic environment.

It is possible that the locale for life's origins was not racemic, even though the global chemical environment contained nearly equal amounts of each pair of stereoisomers. There likely were biases in the inventory of compounds delivered to the Earth by comets and meteorites. For example, some carbonaceous chondrite meteorites contain a significant enantiomeric excess of L-amino acids that are known to be indigenous to the meteorite (Engel and Macko 1997; Cronin and Pizzarello 1997; Pizzarello et al. 2003; Glavin and Dworkin 2009). These in turn could bias terrestrial syntheses, although the level of enantiomeric enrichment generally declines with successive chemical reactions. A special exception are a remarkable set of reactions and fractionation processes that amplify a slight chiral imbalance, even to the level of local homochirality (Kondepudi et al. 1990; Soai et al. 1995; Viedma 2005; Klussmann et al. 2006; Noorduin et al. 2008; Viedma et al. 2008). These systems have in common both a catalytic process for amplification of same-handed molecules and an inhibition process for suppression of opposite-handed molecules.

Some of the most appealing examples of chiral symmetry-breaking reactions involve saturating solutions of various amino acids that form an equilibrium between the liquid phase and solid phase. The solid phase consists of either racemic or enantiopure crystals, and the liquid phase reflects whatever enantiomeric excess exists at the eutectic point for the mixture. For some amino acids, such as serine and histidine, the enantiomeric excess at the eutectic is >90% (Klussmann et al. 2006). This means that, starting from a small concentration imbalance of D- and L-isomers, the imbalance is amplified as both isomers enter the solid phase and the solution phase approaches the eutectic equilibrium. This and related near-equilibrium mechanisms (Noorduin et al. 2008; Viedma et al. 2008) could provide

a means to achieve high enantiomeric enrichment in a local environment. This in turn could bias the production of ribose and the derived nucleotides.

Scientists interested in the origins of life seem to divide neatly into two classes. The first, usually but not always molecular biologists, believe that RNA must have been the first replicating molecule and that chemists are exaggerating the difficulties of nucleotide synthesis. They believe that a few more striking chemical "surprises" will establish that a pool of racemic mononucleotides could have formed on the primitive Earth, and that further experiments with different activating groups, minerals, and chiral amplification processes will solve the enantiomeric cross-inhibition problem. The second group of scientists are much more pessimistic. They believe that the de novo appearance of oligonucleotides on the abiotic Earth would have been a near miracle. Time will tell which is correct.

3.2 Alternative Genetic Systems

The problems that arise when one tries to understand how an RNA World could have arisen de novo on the primitive Earth are sufficiently severe that one must explore other possibilities. What kind of alternative genetic systems might have preceded the RNA World? How could they have "invented" the RNA World? These topics have generated a good deal of speculative interest and some relevant experimental data.

Eschenmoser and colleagues have undertaken a systematic study of the properties of analogs of nucleic acids in which ribose is replaced by some other sugar, or in which the furanose form of ribose is replaced by the pyranose form (Eschenmoser 1999) (Fig. 7B). Strikingly, polynucleotides based on the pyranosyl analog of ribose (p-RNA) form Watson-Crick paired double helices that are more stable than RNA, and p-RNAs are less likely than the corresponding RNAs to form multiple-strand competing structures (Pitsch et al. 1993, 1995b, 2003). Furthermore, the helices twist much more gradually than those of standard nucleic acids, which should make it easier to separate strands of p-RNA during replication. Pyranosyl RNA appears to be an excellent choice as a genetic system; in some ways it seems an improvement compared with the standard nucleic acids. However, p-RNA does not interact with normal RNA to form base-paired double helices.

Most double-helical structures reported in the literature are characterized by a backbone with a six-atom repeat. Eschenmoser and colleagues made the surprising discovery that an RNA-like structure based on threose nucleotide analogs (TNA) (Fig. 7C), although it involves a five-atom repeat, can still form a stable double-helical structure with standard RNA (Schöning et al. 2000). This provides
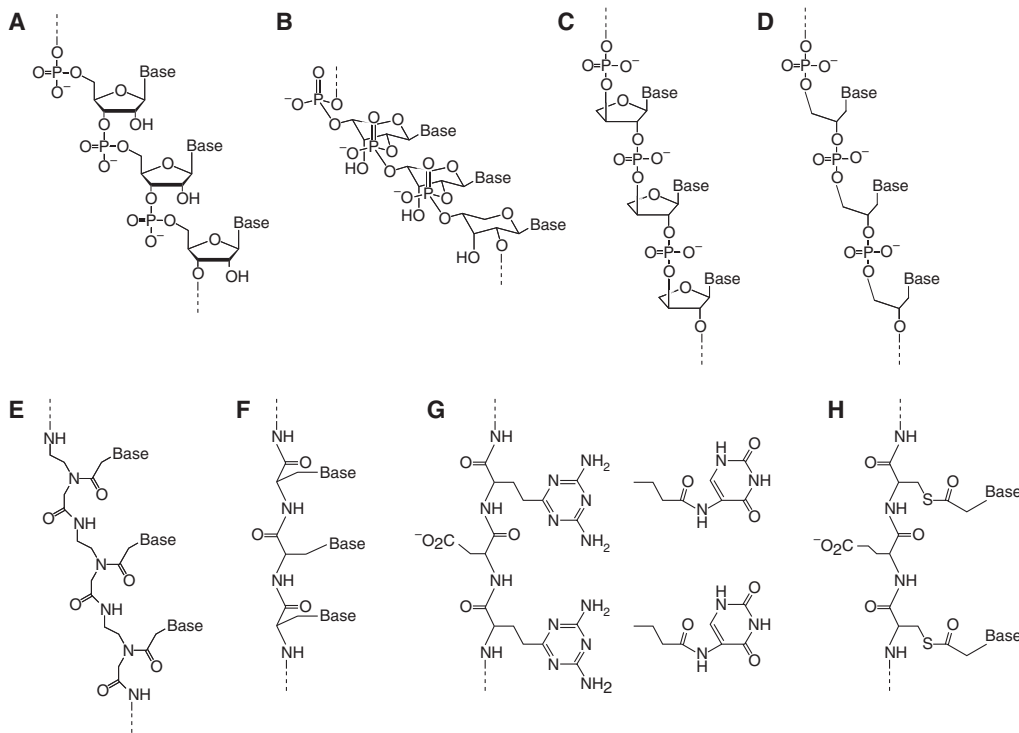
Figure 7. The structures of (*A*) RNA; (*B*) p-RNA; (*C*) TNA; (*D*) GNA; (*E*) PNA; (*F*) ANA; (*G*) diaminotriazine-tagged (*left*) and dioxo-5-aminopyrimidine-tagged (*right*) oligodipeptides; and (*H*) tPNA. ANA contains a backbone of alternating D- and L-alanine subunits. The diaminotriazine tags are shown linked to a backbone of alternating L-aspartate and L-glutamate subunits; the dioxo-5-aminopyrimidine tags (shown unattached) can be linked similarly. tPNA is shown with a backbone of alternating L-cysteine and L-glutamate subunits.

an example of a pairing system based on a sugar that could be formed more readily than ribose: Tetroses are the unique products of the dimerization of glycoaldehyde, whereas pentoses are formed along with tetroses and hexoses from glycoaldehyde and glyceraldehyde. A structural simplification of Eschenmoser's threose nucleic acid has been achieved by Meggers and colleagues (Zhang et al. 2005). They replaced threose by its open chain analogue, glycol, in the backbone of TNA, resulting in glycol nucleic acid (GNA) (Fig. 7D). Complementary oligomers of GNA form antiparallel, double-helices with surprisingly high duplex stabilities.

Peptide nucleic acid (PNA) is another nucleic acid analog that has been studied extensively (Fig. 7E). It was discovered by Nielsen and colleagues in the context of research on antisense oligonucleotides (Egholm et al. 1992, 1993; Wittung et al. 1994). PNA is an uncharged, achiral analog of RNA or DNA in which the ribose-phosphate backbone of the nucleic acid is replaced by a backbone held together by amide bonds. PNA forms very stable double helices with complementary RNA or DNA. Work in the Orgel laboratory has shown that information can be transferred from PNA to RNA, or from RNA to PNA, in

template-directed reactions, and that PNA/DNA chimeras are readily formed on either DNA or PNA templates (Schmidt et al. 1997a,b; Koppitz et al. 1998). Thus it seems that a transition from a PNA World to an RNA World is possible.

The alanyl nucleic acids (ANA) are interesting for a different reason. They are polypeptides formed from nucleo amino acids (Fig. 7F), but pairing structures can be formed only if the two enantiomers of their constituent α-amino acids occur in a regular alternating sequence (Diederichsen 1996, 1997). Because abiotic syntheses of potentially chiral molecules would under almost all circumstances yield racemic products, pairing structures that can be formed from racemic mixtures are particularly attractive. The ANA-type backbone of alternating D- and L-amino acids could, in principle, support paired, double-stranded structures based on a variety of side-chain interactions.

Eschenmoser and colleagues have examined repeating homochiral dipeptide backbones that have either triazines or aminopyrimidines attached at alternating positions (Mittapalli et al. 2007a,b) (Fig. 7G). In this case, not only is the backbone a potential precursor to that of RNA, but also the bases have been replaced by potential precursors,

such as 2,4-diaminotriazine (^{T}NN), 2,4-dioxotriazine (^{T}OO), 2,4-diamino-5-aminopyrimidine (^{AP}NN), and 2,4-dioxo-5-aminopyrimidine (^{AP}OO). Oligomers containing either ^{T}NN or ^{AP}OO subunits were found to pair strongly with complementary RNA, whereas oligomers containing either ^{T}OO or ^{AP}NN subunits did not. Not surprisingly, therefore, pairing between complementary substituted oligodipeptides of the same type (either oligo[^{T}NN]•oligo[^{T}OO] or oligo[^{AP}NN]•oligo[^{AP}OO]) also was weak. However, cross-pairing between oligo(^{T}NN) and oligo(^{AP}OO) was robust (Mittapalli et al. 2007a). This raises the intriguing possibility that an informational polymer could have a mixed composition of ^{T}NN and ^{AP}OO subunits, which would direct the synthesis of an opposing strand that has a complementary sequence as well as a "complementary" backbone composition.

Even more radical are thioester peptide nucleic acids (tPNA) (Fig. 7H), containing a repeating dipeptide backbone with cysteine residues at alternating positions, which are transiently linked via a thioester to a nucleic acid base (Ura et al. 2009). The bases are in dynamic equilibrium between the solution and cysteine positions along the backbone. Occupancy of a base at a particular position is enhanced by the presence of the complementary base on the opposing strand. In this way the informational polymer can self-assemble in a template-directed manner, with mismatched bases exchanging rapidly and matched bases remaining thioesterified for an extended period of time. Perhaps genetic information could be propagated in such a system, although the fidelity of replication, and therefore the maximum number of informational subunits, is likely to be modest.

The studies described previously suggest that there are many ways of linking together the nucleotide bases into chains that are capable of forming base-paired double helices. It is not clear that it is much easier to synthesize the monomers of p-RNA, TNA, GNA, PNA, ANA, or tPNA than to synthesize the standard nucleotides. However, it is possible that a base-paired structure of this kind will be discovered that can be synthesized readily under prebiotic conditions. The properties of the ^{T}NN- and ^{AP}OO-tagged oligodipeptides suggest that it may be fruitful to explore a broader range of potential precursors to RNA, changing the recognition elements as well as the backbone. A strong candidate for the first genetic material would be any informational macromolecule that is replicable in a sequence-general manner and derives from compounds that would have been abundant on the primitive Earth, and preferably has the ability to cross-pair with RNA.

The transition from an RNA-like World to the RNA World could take place in two ways. The transition might be continuous if the pre-RNA template could direct the synthesis of an RNA product with a complementary sequence. Such a transition, for example, from PNA to RNA, would preserve information. RNA could then act as a genetic material in a formerly PNA World. However, even if chimeras were involved in the transition, it is unlikely that the original function of a PNA catalyst could be retained throughout the transition because PNA and RNA have such different backbone structures. A direct and continuous transition from p-RNA to RNA would not be possible because p-RNA does not form complementary double helices with RNA, but this limitation does not apply to TNA and GNA.

The second type of transition can be described as a genetic takeover. A pre-existing self-replicating system evolves, for its own selective advantage, a mechanism for synthesizing and polymerizing the components of a completely different genetic system, and is taken over by it. Cairns-Smith (1982) has proposed that the first genetic system was inorganic, perhaps a clay, and that it "invented" a self-replicating system based on organic monomers. However, he clearly recognized the possibility of one organic genetic material replacing another (Cairns-Smith and Davies 1977). Genetic takeover does not require any structural relationship between the polymers of the two genetic systems. It suggests the possibility that the original genetic system may have been unrelated to nucleic acids.

The hypothesis of a genetic material completely different from nucleic acids has one enormous advantage—it opens up the possibility of using very simple, easily synthesized prebiotic monomers in place of nucleotides. However, it also raises two new and difficult questions. Which prebiotic monomers are plausible candidates as the components of a replicating system? Why would an initial genetic system invent nucleic acids once it had evolved sufficient synthetic know-how to generate molecules as complex as nucleotides?

A number of prebiotic monomers that might have made up a simple genetic material have already been suggested. They include hydroxy acids (Weber 1987), amino acids (Orgel 1968; Zhang et al. 1994), phosphomonoesters of polyhydric alcohols (Weber 1989), aminoaldehydes (Nelsestuen 1980), and molecules containing two sulfhydryl groups (Schwartz and Orgel 1985). The list could be expanded almost indefinitely. The discussion here concerns a small class of these monomers that appear to be particularly attractive in the light of recent work on enzyme mechanisms.

There is accumulating evidence that several enzymes that make or break phosphodiester bonds have two or three metal ions at their active sites (Cooperman et al. 1992; Sträter et al. 1996). In the case of the editing site for phosphodiester hydrolysis in the Klenow fragment of *Escherichia coli* DNA polymerase I, no other functional groups

of the enzyme come close to the phosphodiester bond that is cleaved. This has led to the suggestion that the major role of the enzyme is to act as scaffolding on which to hang metal ions in precisely determined positions (Beese and Steitz 1991; Steitz 1998). A similar suggestion has been made for ribozymes on the basis of both indirect and direct evidence (Freemont et al. 1988; Yarus 1993; Steitz and Steitz 1993; Shan et al. 1999, 2001; Stahley and Strobel 2005).

Perhaps these observations can be extended to suggest that, if informational polymers preceded RNA, they may also have been dependent on metal ions for their catalytic activity. If so, the range of prebiotic monomers that needs to be considered is greatly reduced. In addition to the functional groups that react to form the backbone, the monomers must have carried metal-binding functional groups. If the metal ions involved were divalent ions such as Mg^{2+} and Ca^{2+}, the side groups are likely to have been carboxylate or phosphate groups. If transition metal ions were involved, sulfhydryl groups and possibly imidazole derivatives are likely to have been important.

Prebiotic monomers suitable for building polymers that bind Mg^{2+} or Ca^{2+} include aspartic acid, glutamic acid, and serine phosphate among biologically important amino acids. ß-amino acids, such as isoglutamic acid, hydroxydicarboxylic acids, such as α-hydroxysuccinic acid, and hydroxytricarboxylic acids, such as citric acid, are other possible candidates. A polymer containing D-aspartic acid, L-aspartic acid, and glycine as its subunits is typical of potentially informational co-polymers that might, in the presence of divalent metal ions, both replicate and function as a catalyst. Transition-metal ions might play a corresponding role for polymers containing cysteine or homocysteine. The present challenge is to show replication, or at least information transfer in template-directed synthesis, in some such system.

What selective advantage could a simpler, metabolically competent system derive from the synthesis of oligonucleotides? This is a baffling question. Most arguments that come to mind do not stand up to detailed analysis. If, for example, one postulates that nucleotides were first synthesized as parts of cofactors such as DPN, one must explain why the particular heterocyclic bases and sugars were chosen. Even if one supposes that among the many "experiments" in secondary metabolism performed by early organisms one happened by accident on a pair of complementary nucleotides that could form a replicating polymer, one must still explain how polymerization subsequently contributed to the success of the "inventor." Could oligonucleotides, by hybridization, have functioned at first as selective "glues" for tying pairs of macromolecules together? Could RNA have been invented by one organism as "antisense" against the genome of another?

The discussion so far, even though highly speculative, is still conservative in overall outlook. It supposes that the original information-accumulating system that led to the evolution of life on Earth was either RNA or some linear copolymer that replicated in an aqueous environment in much the same way as RNA. There remains a lingering doubt that the discussion is not on the right track at all; maybe the original system was not an organic copolymer (Cairns-Smith 1982), or maybe it replicated in a nonaqueous environment and RNA is an adaptation that permitted invasion of the oceans. Perhaps systems of high complexity can develop without any need for a genome in the usual sense (Dyson 1982; Kauffman 1986; Wächtershäuser 1988; De Duve 1991; Eschenmoser 2007). Perhaps . . .

Laboratory simulations of prebiotic chemistry are dependent on organic chemistry and can only explore the kinds of reactions understood by organic chemists. A good deal is known about reactions in aqueous solution, but less about reactions at the interface between water and inorganic solids. Very little is known about reactions in systems in which inorganic solids are depositing from aqueous solutions containing organic material. It is hard to see how speculative schemes involving heterogeneous aqueous systems can be tested until much more is known about the underlying branches of chemistry.

4   CONCLUDING REMARKS

After contemplating the possibility of self-replicating ribozymes emerging from pools of random polynucleotides and recognizing the difficulties that must have been overcome for RNA replication to occur in a realistic prebiotic soup, the challenge must now be faced of constructing a realistic picture of the origin of the RNA World. The constraints that must have been met in order to originate a self-sustained evolving system are reasonably well understood. One can sketch out a logical order of events, beginning with prebiotic chemistry and ending with DNA/protein-based life. However, it must be said that the details of this process remain obscure and are not likely to be known in the near future.

The presumed RNA World should be viewed as a milestone, a plateau in the early history of life on Earth. So too, the concept of an RNA World has been a milestone in the scientific study of life's origins. While this concept does not explain how life originated, it has helped to guide scientific thinking and has served to focus experimental efforts. Further progress will depend primarily on new experimental results, as chemists, biochemists, and molecular biologists work together to address problems concerning molecular replication, ribozyme enzymology, and RNA-based cellular processes.

ACKNOWLEDGMENTS

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