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The roads to and from the RNA world

Jason P. Dworkin[a], Antonio Lazcano[b,*], Stanley L. Miller[c]

[a] Laboratory for Extraterrestrial Physics, Code 691, NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA
[b] Facultad de Ciencias, Univ. Nacional Autonoma de Mexico, Apdo. Postal 70-407, Cd. Universitaria, Mexico D.F. 04510, Mexico
[c] Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0506, USA

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Abstract

The historical existence of the RNA world, in which early life used RNA for both genetic information and catalytic ability, is widely accepted. However, there has been little discussion of whether protein synthesis arose before DNA or what preceded the RNA world (i.e. the pre-RNA world). We outline arguments of what route life may have taken out of the RNA world: whether DNA or protein followed. Metabolic arguments favor the possibility that RNA genomes preceded the use of DNA as the informational macromolecule. However, the opposite can also be argued based on the enhanced stability, reactivity, and solubility of 2-deoxyribose as compared to ribose. The possibility that DNA may have come before RNA is discussed, although it is a less parsimonious explanation than DNA following RNA.
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1. Introduction

One of the most important questions facing the study of the origin of life is the nature of the first genetic material. It is usually stated that the transition from non-life to life occurred when this genetic material began to accumulate and replicate in the primitive environment. The importance of RNA in the origin of life has been asserted by so many researchers for so many years that it has been generally assumed that RNA was among the first informational polymers (see for example, Belozerskii, 1959; Brachet, 1959; Oparin, 1961; Rich, 1962; Buchanan, 1965; Haldane, 1965; Woese, 1967; Crick, 1968; Orgel, 1968; Kuhn, 1972; Eigen and Schuster, 1979; White III, 1982).

The RNA world is a hypothetical period of the early biosphere when both the information needed for life and the enzymatic activity of living organisms were contained in RNA molecules (Gilbert, 1986; Joyce, 2002). This proposal stems from the discovery of catalytic activity in RNA (Cech et al., 1981; Guerrier-Takada et al., 1983), and it has often been cited as the solution to the problem of whether life first arose as DNA or protein. The RNA world and its catalytic repertoire have been extensively discussed (Orgel, 1986; Beaudry and Joyce, 1992; Piccirilli et al., 1990; Szostak and Ellington, 1993; Ellington, 1994; Gesteland and Atkins, 1993; Joyce, 1998, 2002). There has been some discussion about the transition from the pre-RNA world into the RNA world (Orgel, 1986, 1989; Schwartz et al., 1987; Joyce, 1989; de Duve, 1993; Piccirilli, 1995; Miller, 1997). The possibilities of a simultaneous origin of RNA and DNA (Oró and Stephen-Sherwood, 1974) or of DNA before proteins (Benner et al., 1987, 1989, 1993) have also been addressed. As summarized by Kumar and Yarus (2001) there is an increasing amount of experimental evidence suggesting that protein synthesis evolved in an RNA world. However, very little has been said about the transition from the RNA world to the modern world of DNA/RNA/protein (where DNA stores the information, RNA serves auxiliary functions, and protein does the catalysis). The primary focus has been based on the observations on the importance of RNA, including the RNA primer involved in DNA

*Corresponding author. Tel.: +52-5-56224823; fax: +52-5-561660451.
E-mail addresses: jason.dworkin@nasa.gov (J.P. Dworkin), alar@hp.fciencias.unam.mx (A. Lazcano), smiller@ucsd.edu (S.L. Miller).

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replication, the assumption of the lateness of DNA as a more stable archive of genetic information (Ferris and Usher, 1983; Lazcano et al., 1988a, b, 1992; Benner et al., 1989; Poole et al., 2000), and the study of the evolution of ribonucleotide reductases as an essential step in the transition from the RNA to the extant DNA/RNA/protein world (Follmann, 1982; Harder, 1993; Reichard, 1993; Freeland et al., 1999).

The hypothesis that the RNA world, which may have been preceded by simpler living entities, eventually evolved into the DNA/protein world which had all the characteristics of modern biochemistry, is currently the most favored one. However, there are other alternatives. The purpose of this paper is to discuss these other possibilities. In this paper we address the possibility that deoxyribose came before ribose, and then whether DNA came before protein synthesis or the reverse. Other aspects of this question have been discussed by Freeland et al. (1999). We also examine the various ways to shift from the pre-RNA world to the RNA world, including the possibility that DNA came before RNA. Although we agree that the most parsimonious interpretation of the available evidence favors the precedence of RNA over proteins and DNA, it is also true that evolution does not always follow the straightest course. The discussion presented here is clearly speculative, but it is hoped that it will be a guide for further experiments.

2. The prebiotic availability of deoxyribose

Are there any arguments that could be used to favor the existence of a DNA world (devoid of proteins) over the most familiar RNA world? The prebiotic synthesis of deoxyribose from glyceraldehyde and acetaldehyde is poor (Oró and Cox, 1962), but the prebiotic synthesis of ribose is not vastly better (Shapiro, 1988). There are other potential prebiotic pathways being explored for the synthesis of ribose from small phosphorylated aldehydes in the presence of hydroxide minerals under neutral conditions (Krishnamurthy et al., 1999), but equivalent pathways to 2-deoxyribose have not been studied. Although sugars are currently out of favor as prebiotic reagents, the presence of sugar acids, including both ribosugar- and deoxysugar acids in the 4.6×10^9 years old Murchison meteorite suggest that they may have been present in the primitive Earth, derived from the accretion of extraterrestrial sources (Cooper et al., 2001) or from endogenous processes involving formaldehyde and its derivatives.

It has been argued (Robertson and Miller, 1995; Robertson et al., 1996) that drying lagoon conditions could have acted as a prebiotic reactor. The solubility of 2-deoxyribose is 30 molal (Dworkin, 1997) while ribose is 20 molal (Goldberg and Tewari, 1989) at 25°C. The greater solubility of 2-deoxyribose would be a slight advantage in a drying scenario for the synthesis of nucleosides or their precursors (Fuller et al., 1972a, b). 2-Deoxyribose may have been more reactive under prebiotic conditions: for example it reacts about 150 times faster than ribose with the alternative base urazole to form the nucleoside at 25°C (Dworkin and Miller, 2000). In addition, Larralde et al. (1995) have shown that 2-deoxyribose decomposes 2.6 times more slowly than does ribose at 100°C. Other advantages of DNA over RNA are that it has one fewer chiral center, has greater stability at the pH of the current ocean (8.2), and does not has the 2′5′ and 3′5′ ambiguity in polymerizations.

Many origin of life researchers interested in carbohydrates have focused on ribose on the assumed existence of an RNA world which pulled its ribose from the prebiotic soup. As a result there have been very few studies on prebiotic sources of 2-deoxyribose or on the synthesis of this and other sugars via primitive biocatalysts. The possibility that deoxyribose derivatives played a role in early biological evolution following the pre-RNA world deserves further attention. Thus, prebiotic and early biosynthetic pathways for deoxyribose synthesis are as important to investigate as those for ribose (Dworkin and Miller, 1996).

3. How to get out of the RNA world

It is generally assumed that the RNA world lasted for a relatively small period of time, but alternative viewpoints have been proposed (Benner and Ellington, 1987; Benner et al., 1987). This is primarily due to the conjunction of the presumed inefficiency of primitive ribozymes combined with the instability of RNA (Woese, 1967; Miller and Orgel, 1974) as well as its subunits (especially ribose) (Larralde et al., 1995). With the exception of low temperatures, no mechanism has been discovered that could enhance the stability of RNA and its components under prebiotic conditions. However, there is an increasing large amount of empirical evidence demonstrating the versatility and ample catalytic repertoire of ribozymes. Although it has been claimed that RNA viruses may be the last remnants of the RNA world (Maizels and Weiner, 1994), it is clear that the latter no longer exists. Thus, it is important to ask what evolutionary pathway life took from it to the modern world of DNA/RNA/protein.

If we assume that the transition from the RNA world into the modern world took place through small evolutionary steps, then there are at least two possible pathways out of the RNA world to consider. These pathways lead to DNA as the informational molecule with RNA as the catalyst, or to RNA as the informational molecule with protein as the catalyst. This is illustrated in Fig. 1. The path out of the RNA world

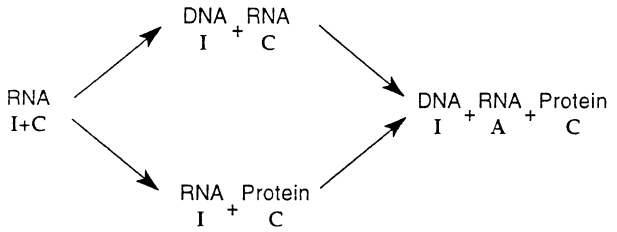

Fig. 1. Pathways out of the RNA world. Each arrow is a genetic or catalytic takeover event consisting of the addition or loss of a new informational or catalytic polymer. A molecule can fulfill two roles (such as RNA in the RNA world), but we will assume that a role cannot be fulfilled by two different molecular species simultaneously. The RNA world is designated by RNA (I + C), i.e. information + catalysis while the modern world uses RNA as an auxiliary molecule (A). The duration of each system is unspecified.

would depend on the properties of this RNA. For example, if it contained extensively modified bases (cf. Robertson and Miller, 1995; Poole et al., 2000) and had its catalytic repertoire enhanced by cofactors (Benner et al., 1987, 1989), then it is reasonable that DNA could have acted as a clean storehouse for genetic information. This would lead to the top pathway in Fig. 1. It is unlikely that RNA had to rely solely on the 2′-hydroxyl as the primary catalytic moiety; the chemical functionality and the breadth of ribozyme catalysis could have resulted from modified ribonucleotides, different cofactors, and amino acids and peptides. The possibility of porphyrin-dependent ribozymes that could catalyse the reduction of ribonucleotides has been raised (Benner et al., 1989). It has been thought that radical chemistry is outside the repertoire of ribozymes (Freeland et al., 1999), but many nucleotide-like coenzymes are known to facilitate reactions in which organic radicals take part (Frey, 2001). Selection of small RNA aptamers capable of binding cyanocobalamin (vitamin B_{12}) (Lorsch and Szostak, 1994; Sussman et al., 2000) opens the possibility their participation in radical catalysis. Alternative mechanisms, such as the ribozyme-mediated reduction of an attached purine followed by acid-catalysed elimination of the ribose 2′-OH group, are also conceivable (Albert Eschenmoser, pers. comm.). In both cases, the bottom pathway of Fig. 1 would have been followed.

4. DNA before protein synthesis: was there a DNA/RNA world?

A strong argument in favor of DNA before protein synthesis can be made on the basis of simplicity. Transcription is simple compared to translation, even for a minimal protein system, so it is likely that either a few mutations could change a primitive RNA polymerase into an early reverse transcriptase or the early polymerases were non-specific toward the nature of their template and nucleotide substrates (Lazcano et al., 1988b). Combining catalytic and information functions in similar molecules (RNA and DNA) could have allowed for an easy transition for early organisms (Fig. 1). The optimization of these functions is best handled with separate molecules once the newly formed DNA/RNA organisms had established themselves.

In order to get fidelity adequate for a multigene genome, it is probably necessary to have some repair mechanisms. We envision the straightforward recognition of DNA by RNA (as compared to RNA by protein) as being a much less complex method of recognizing misincorporations or the products of hydrolytic reactions.

If the ribozymes are highly modified for catalytic efficiency, or if the tertiary structure of the highly evolved ribozyme is very stable, then replication may be difficult and prone to error, leading to the separation between replicative DNA and catalytic RNA. The possibility of catalytic RNA-mediating DNA replication has been raised (Brewin, 1972). This could have given rise to what are believed to be metabolic fossils of the RNA world, such as the biosyntheses of histidine (White III, 1982), nicotinamide (Cleaves and Miller, 2001), as well as CoA, NAD, and FAD (Benner et al., 1987, 1989; Huang et al., 2000; Jadhav and Yarus, 2002). Although in some cases deoxyribozymes can be better catalysts than ribozymes (Breaker, 1997; Santoro and Joyce, 1997; Ellington and Robertson, 1999), DNA is considered to be less inclined to form complex secondary structures and is, in general, a less-efficient catalyst (Cuenoud and Szostak, 1995), so it is less likely to miscode and is thus a more stable medium for storing genetic information (Bashkin, 1997). For example, a structural comparison of $tDNA^{Phe}$ with $tRNA^{Phe}$ showed that while the double-stranded regions of DNA adopted a more B-like conformation, the overall tertiary structure of the two molecules is probably similar (Lim and Barton, 1993).

Both DNA and RNA are stable in the duplex form (Usher and McHale, 1976), but at low temperatures and high salt concentrations the RNA:RNA duplex is more stable than its DNA:DNA counterpart, with the DNA:RNA double helix being the least stable combination (e.g. Oró and Stephen-Sherwood, 1974). In general, however, DNA is considered more stable than RNA (Woese, 1967; Miller and Orgel, 1974), unless the early biotic environment was strongly acidic, so it is a better storehouse of genetic information. If ribo-organisms did not have a sufficiently fast turnover rate, then RNA instability would be a major cause of loss of genetic information in the RNA world. This applies particularly to RNA in the presence of divalent cations (Eichhorn et al., 1971; Brown et al., 1983). Even more important is

the need for double-stranded nucleic acids for replication error correction and repair of hydrolytic damage. In addition, the rate of hydrolysis is slower in double-stranded DNA, a factor of 200 in the case of cytosine (Lindahl, 1982).

As discussed above, the possibility of a ribozymic ribonucleotide reductase has been raised (Benner et al., 1989). Another mechanism generating a DNA/RNA world involves the simultaneous prebiotic syntheses and accumulation of ribonucleotides and 2′-deoxyribonucleotides and their polymerization products (Oró and Stephen-Sherwood, 1974). Although this possibility is currently out of favor, if reasonable prebiotic syntheses of RNA are discovered it is likely that they would apply equally well to DNA. Such hypothetical syntheses would be complicated by the diversity of chemicals in the prebiotic environment, so a mixture of ribo- and deoxyribonucleotides would have been simultaneously available.

5. Protein synthesis before DNA: was there a RNA/protein world?

Although Schuster (1993) suggested the presence of a "protein-assisted RNA world", in which the pure RNA world is augmented by a suite of protein enzymes (the source of these enzymes is not clear), most authors have assumed that an RNA/protein world follows the RNA world. This assumption is based on the observations of Ferris and Usher (1983) and Lazcano et al. (1988a, 1992) listed in Table 1. Three of the arguments listed in Table 1 can apply to DNA/RNA as well as DNA/protein (7, 8, and 10). Others do not address whether protein came before DNA, only the ancient nature of RNA (3, 6, and 9). Two have been investigated and are no longer compelling evidence [1 (Oró and Cox, 1962) and 2 (reviewed by James and Ellington, 1995)]. Reason 5 assumes that 2′–3′-cyclic phosphates were prebiotic compounds, and this has yet to be demonstrated. Reason 4 seems inverted: early genetic material would be expected to have been more stable because protection and repair enzymes were not present.

Table 1

1	The presumed lack of prebiotic deoxyribose
2	Activated ribonucleotides polymerize more readily than activated deoxyribonucleotides
3	The biosynthesis of deoxyribonucleotides is through ribonucleotides
4	RNA is less stable than DNA, and is thus more ancient
5	Ribonucleoside-2′,3′-cyclic phosphate is a potential prebiotic activated nucleotide
6	Protein biosynthesis depends on different RNAs, not from DNA
7	RNA can store genetic information
8	RNA can be a catalyst
9	Ribosides are subunits of many coenzymes, some of which have prebiotic syntheses
10	The reactive potential of the 2′OH of ribosides

There is a distinct advantage in keeping the genetic material separate from the catalytic molecule, both physically and in type of molecule, i.e. in achieving the separation of genotype and phenotype. As summarized by Kumar and Yarus (2001), several other arguments can be made in favor of an RNA/protein world following the RNA world. Another argument in favor of proteins before DNA is that the biosynthesis of protein enzymes is more energy efficient than ribozymes (Crick, 1968). This should be particularly relevant if there are sufficient abiotically generated amino acids in the environment and if the ribonucleotides need to be synthesized.

There are several indications that the first protein synthesis system was much simpler (Ban et al., 2000; Nissen et al., 2000). The present complexity arises from the necessity for high fidelity, which may not have been an initial requirement. Thus a small, simple, and error-prone ribosome could have developed to synthesize proteins that would be short and of low specificity and activity (Woese, 1965). Even in this case, however, the amount of information in a simple translation system involving only few ancestral tRNA genes and catalytic RNAs is still overwhelming compared to an unspecific polymerase and ribonucleotide reductase system.

6. How to get into the RNA world

The concept of an RNA world is based on both the catalytic repertoire of ribozymes (Cech et al., 1981; Guerrier-Takada et al., 1983; Landweber et al., 1998), including a catalytic RNA with RNA replicase activity (Johnston et al., 2001) and on observations of contemporary metabolism (Lazcano et al., 1988a; Joyce, 1989, 2002; Ellington, 1993). However, current biosynthetic pathways could have easily overprinted clues to earlier metabolisms, and the existence of an earlier self-replicating molecule precludes the necessity of an (all) RNA world. There still could have been an RNA world, but it is not necessary in the progression from pre-RNA to the modern system of DNA/protein assisted by RNA, it is merely necessary that RNA was involved in information or catalysis after pre-RNA played such a role.

The possible progressions from pre-RNA to today are shown in Fig. 2, with the assumptions that only pre-RNA, RNA, DNA, and protein are involved with the same definitions and assumptions used in Fig. 1. This figure is overwhelming; one can, however, make assumptions to eliminate unlikely pathways. For

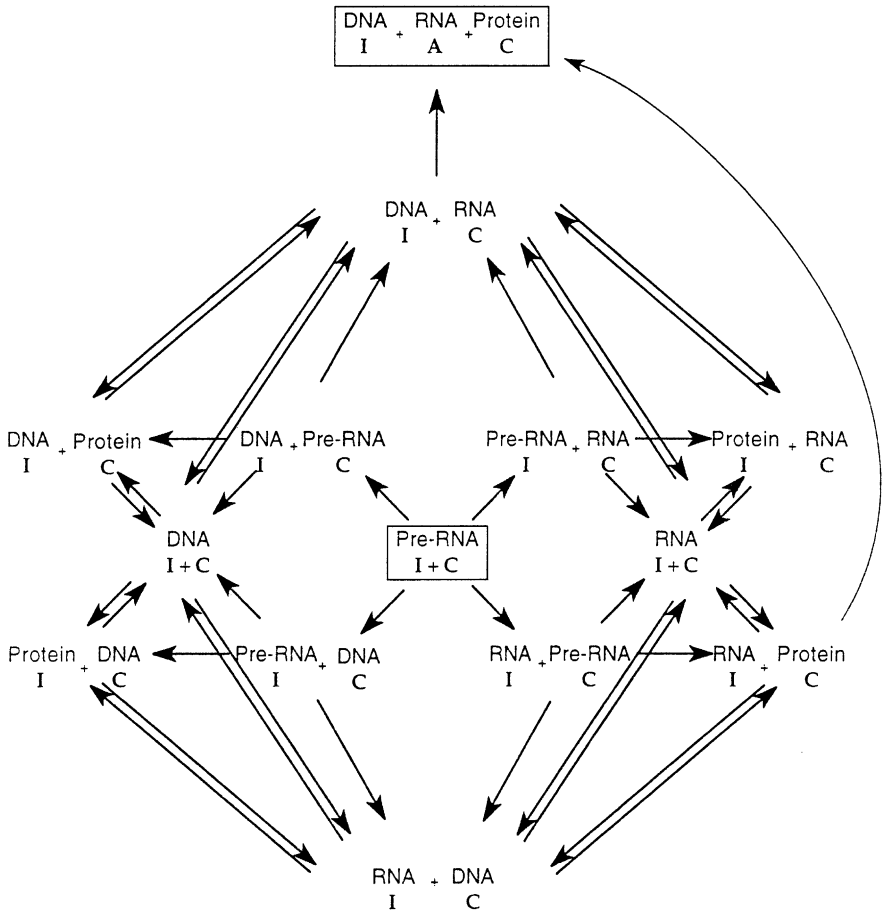

Fig. 2. Possible pathways out of the pre-RNA world (boxed in the center) into the modern world (boxed at top) using the conventions and assumptions of Fig. 1, including the hypothesis that once the pre-RNA world is lost it can never be used.

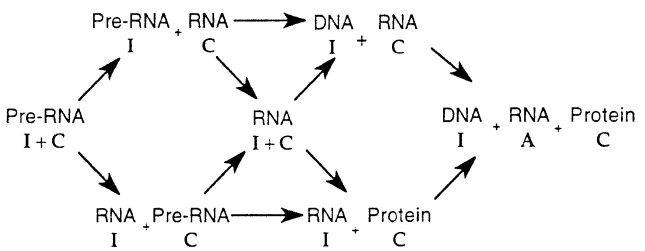

Fig. 3. RNA-first pathways out of the pre-RNA world using the conventions of Fig. 1 and the assumptions in the text.

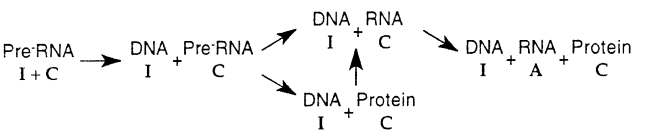

Fig. 4. DNA-first pathways out of the pre-RNA world using the conventions of Fig. 1 and the assumptions in the text.

example, three assumptions greatly simplify the figure. First, the penultimate system involved RNA in some capacity. This is supported by the metabolic arguments of the importance of RNA (Lazcano et al., 1988a, 1992; Joyce, 1989, 2002). Second, although DNA enzymes (deoxyribozymes) have been made (Breaker and Joyce, 1994, 1995; Cuenoud and Szostak, 1995), they may have not developed naturally. Third, protein is an inadequate informational macromolecule (Miller and Orgel, 1974). This reduces the possible routes out of the pre-RNA world to two possible pathways, RNA first (Fig. 3) or DNA first (Fig. 4).

The RNA first pathways (Fig. 3) can lead to the DNA/RNA or RNA/protein worlds via the RNA world. However, evolution could have skipped the RNA world in favor of either pre-RNA/RNA or RNA/pre-RNA worlds. The pre-RNA/RNA pathway has essentially been suggested by Nielsen (1993) with the proposal of a PNA/RNA world. Work on nucleic acid analogs suggests that a wide variety of macromolecules are possible even when restricted to sugar–phosphate backbones (Eschenmoser, 1994; Schoning et al., 2000). This would alter the previous discussion of the RNA/protein versus DNA/RNA worlds depending on the chemical properties of pre-RNA. Fig. 4 shows DNA coming directly from pre-RNA. This raises the question of whether DNA could have preceded RNA?

7. Which is more ancient: DNA or RNA?

Woese (1967) first suggested that DNA may have been more abundant in the prebiotic environment than RNA due to the greater stability of DNA in mildly basic conditions, postulated to have been caused primarily by an ammonia-rich ocean and by the weathering of basic rocks.

In addition to the replicative ability, catalytic properties, and the central role in biochemistry of RNA, the existence of the RNA world has been based on several metabolic arguments (Eigen et al., 1981; White III, 1982; Ferris and Usher, 1983; Lazcano et al., 1988a, b, 1992; Joyce, 1989; Ellington, 1993; Schwartz, 1993; James and Ellington, 1995; Bloch, 1996) such as the biosyntheses of histidine, deoxyribonucleotides, and deoxythymidine. However, none of these are compelling reasons for the precedence of RNA over DNA. DNA can certainly act as a template for its replication and with the experimental development of deoxyribozymes, DNA has joined the ranks of catalytic species. No natural DNA enzymes have been described, perhaps because the early appearance of ribozymes diminished the likelihood of additional catalytic nucleic acids. The importance of DNA in cellular function as the repository of genetic information could be interpreted as a central and thus "ancient" biological process. While RNA is more versatile and performs more of these functions [e.g. RNA is a primer for DNA replication (Eigen et al., 1981) and is central to ribosome function (Noller et al., 1992; Ban et al., 2000; Nissen et al., 2000)], it can only be said that RNA played an important role before the last common ancestor (Tekaia et al., 1999; Delaye and Lazcano, 2000; Lazcano Araujo, 2001; Anantharaman et al., 2002), but it cannot be said what preceded it.

The presence of ribose as a component of many coenzymes is a powerful argument for the importance of RNA monomers and dimers early in evolution. It cannot be said if these metabolic fossils are remnants of excised genetic or catalytic material. While coenzymes can be viewed as molecular fossils of ancient metabolic systems, they are not necessarily fossils of the *first* metabolic system. Since each genetic takeover at least partially overprints pathways from the previous system, coenzymes are more likely remnants of the system just before DNA/protein than of earlier pre-RNA systems. This argument holds not only for molecular fossils and for biosynthetic pathways, but also for DNA replication, translation, and all other cellular mechanisms.

The biosynthetic arguments are entirely derived from complicated modern metabolic pathways. These pathways could have easily overprinted older ones as environmental conditions around these ancient organisms changed. While it has been stated that recent features of metabolism are superimposed on remnants of ancient life (Benner et al., 1989), the overprinted metabolism may obscure or obliterate the message of the previous pathway. Of course, if the metabolic pathways evolved backwards (Horowitz, 1945) then the biosynthesis of 2-deoxyribose from ribose would suggest that RNA came from DNA (Ferris and Usher, 1983).

One would expect that even the primitive pre-RNA catalysts would be more efficient and selective than any abiotic chemistry going on around them. However, these early catalysts were probably much slower and less selective than modern enzymes. Thus, ideas of what ingredients could be used in early life shifts from ease of synthesis to ease of incorporation, versatility, stability, and reactivity.

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