



## 1 An interpretive review of the origin of life research

2 DAVID PENNY\*

3 *Allan Wilson Center for Molecular Ecology and Evolution, Massey University, Palmerston North,*  
4 *New Zealand; \*Author for correspondence (e-mail: d.penny@massey.ac.nz)*

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8 **Abstract.** Life appears to be a natural property of matter, but the problem of its origin only arose  
9 after early scientists refuted continuous spontaneous generation. There is no chance of life arising  
10 'all at once', we need the standard scientific incremental explanation with large numbers of small  
11 steps, an approach used in both physical and evolutionary sciences. The necessity for considering  
12 both theoretical and experimental approaches is emphasized. After describing basic principles that  
13 are available (including the Darwin-Eigen cycle), the search for origins is considered under four  
14 main themes. These are the RNA-world hypothesis; potential intermediates between an RNA-  
15 world and a modern world *via* the evolution of protein synthesis and then of DNA; possible  
16 alternatives to an RNA-world; and finally the earliest stages from the simple prebiotic systems to  
17 RNA. The triplicase/proto-ribosome theory for the origin of the ribosome is discussed where triples  
18 of nucleotides are added to a replicating RNA, with the origin of a triplet code well-before protein  
19 synthesis begins. The length of the code is suggested to arise from the early development of a  
20 ratchet mechanism that overcomes the problem of continued processivity of an RNA-based RNA-  
21 polymerase. It is probable that there were precursor stages to RNA with simpler sugars, or just two  
22 nucleotides, but we do not yet know of any better alternatives to RNA that were likely to arise  
23 naturally. For prebiotic stages (before RNA) a flow-reactor model is suggested to solve metabo-  
24 lism, energy gradients, and compartmentation simultaneously – thus the intense interest in some  
25 form of flow reactor. If an autocatalytic cycle could arise in such a system we would be major steps  
26 ahead. The most likely physical conditions for the origin of life require further clarification and it is  
27 still unclear whether the origin of life is more of an entropy (information) problem (and therefore  
28 high temperatures would be detrimental), rather than a kinetic problem (where high temperatures  
29 may be advantageous).

### 30 Is the origin of life solvable scientifically?

31 Consider the following experiment. Take cysts (dried fertilized eggs) of the  
32 common brine shrimp (*Artemia*) – a multicellular Crustacean of considerable  
33 complexity. Place the cysts in a small capillary tube, freeze them, transfer them  
34 to liquid helium at a temperature below 2.2 °K (less than –270 °C) and leave  
35 6 days at this almost absolute zero temperature. Then slowly warm them up to  
36 room temperature and see whether the cysts survive, hatch, eat, grow, develop  
37 (with a functioning brain and nervous system) and reproduce. They do (see  
38 Skoultschi and Horowitz 1964).

39 At absolute zero, although the information about the positions of atoms  
40 within chemicals is retained, virtually all prior information about electron  
41 velocity (speed and direction) and distribution in energy orbitals is lost.

42 Therefore, because of the brine shrimp example, life appears to be a natural  
43 consequence of a specific chemical organization of matter; in principle the  
44 origin of life is solvable. It should be possible to recreate life, but it certainly  
45 will not be easy. It should only require clutches of chemists, tapping at type-  
46 writers, designing amino acid and nucleic acid sequences to synthesize and  
47 assemble inside a membranous vesicle, along with the right combination of  
48 ions and small molecules. When done right, the new organism will start  
49 growing and reproducing – to a biologist this is *coito ergo sum*.

50 There were alternatives, for example, that Life (with a capital L) had  
51 properties additional to the orderings of its chemicals. It is logically possible  
52 that even when all the chemicals were arranged in the correct order, that some  
53 special combination of rotational and vibrational velocities, plus distributions  
54 of electrons in orbitals, had to be given externally; only then did this combi-  
55 nation become self-sustaining Life. This would be a scientific version of a ‘vital  
56 principle’ of Life. A useful analogy (Morowitz, pers. comm. 1962) is a jellyfish  
57 that has a circular nerve around its periphery. Stimulating the nerve sends an  
58 action potential both ways around the jellyfish, which cancel each other out  
59 when they meet. But it is relatively easy to prematurely stop one action po-  
60 tential (a temporary lowering of nerve temperature with a piece of ice will do).  
61 The other potential just keeps rolling along; round and round, round and  
62 round the circular nerve. The basic state was there in the structure and phys-  
63 iology of the nerve of the jellyfish, but it needed an external stimulus to get the  
64 potential rolling. Perhaps that was what Life needed, all chemicals in the right  
65 arrangement, then a special kick-start to get Life going. However, if this were  
66 the case, compare our brine shrimp and jellyfish experiments. If jellyfish could  
67 survive to absolute zero and back, the rolling action potential would have been  
68 lost; the system would only go back to the non-living ground state before  
69 receiving the kick-start.

70 Thus the actual experiment of taking *Artemia* down to around absolute zero,  
71 with it still surviving, eliminates some alternative models – distinguishing life  
72 from Life. To be more cautious, the experiment does not ‘prove’ that life is  
73 completely defined by chemicals and their ordering; that requires actually  
74 assembling all the chemicals in the proverbial test-tube. What we conclude  
75 from the Morowitz experiment is that it is reasonable to aim to recreate life  
76 *de novo*. In contrast, there are many possible experiments that are not sensible  
77 to try, such as training a cow to jump over the moon. And in demonstrating  
78 that life appears to be a property of organized matter, we have not even given a  
79 formal definition of life – though it will be helpful later to look at the  
80 components we expect in a living system.

### 81 **Early scientists find the problem**

82 It was part of European indigenous knowledge that life continued to arise  
83 spontaneously. For example,

84 *'Captain Lancaster, in his voyage in 1601, narrates that on the sea sands of*  
 85 *the Island of Sombrero, in the East Indies, he 'found a small twig growing*  
 86 *up like a young tree, and on offering to pluck it up it shrinks down into the*  
 87 *ground, and sinks, unless held very hard. On being plucked up, a great worm*  
 88 *is found to be its root, and as the tree groweth in greatness, so does the*  
 89 *worm diminish; and as soon as the worm is entirely turned into a tree it*  
 90 *rooteth in the earth, and so becomes great. This transformation is one of the*  
 91 *strangest wonders that I saw in all my travels: for if this tree is plucked up,*  
 92 *while young, and the leaves and bark stripped off, it becomes a hard stone*  
 93 *when dry, much like white coral: thus is this worm twice transformed*  
 94 *different natures. Of these we gathered and brought home many.'* Under-  
 95 lining added, (extract from Charles Darwin, Voyage of the Beagle. 1840,  
 96 p. 106)

97 Captain Carpenter (in 1601) observed changes between 'animal, vegetable  
 98 and mineral'. The indigenous view in Europe (and almost certainly in other  
 99 parts of the world) was that 'kinds' or 'forms' of plants and animals continued  
 100 to arise by spontaneous generation. It was accepted that there was continued  
 101 interconversion between living forms, and between living and non-living  
 102 matter. After all, if a caterpillar could turn into stone (a chrysalis!), which then  
 103 turns into a butterfly, why couldn't a barnacle turn into a goose, or a plant into  
 104 an insect? The Old Testament provided support for continued spontaneous  
 105 generation

106 *'And God said, Let the earth bring forth grass, the herb yielding seed, and*  
 107 *the fruit tree yielding fruit after his kind whose seed is in itself, upon the*  
 108 *earth: and it was so',*  
 109 *'And God said, Let the earth bring forth the living creature after his kind,*  
 110 *cattle, and creeping thing, and beast of the earth after his kind, and it was*  
 111 *so'. Genesis 1:11 & 24*

112 This was interpreted as continued spontaneous generation, spontaneous  
 113 generation did not occur just once thousands of years ago, it happened again,  
 114 and again, and again. Indeed it was a heresy in Europe during the Middle Ages  
 115 not to accept that spontaneous generation still continued, a modern creationist  
 116 would have been a medieval heretic. To deny the Creator the power to make  
 117 life continuously would be an affront, fully justifying being burnt at the stake  
 118 (though presumably one would have to be without sin in order to light the first  
 119 match). Similarly, there is no reference here to 'species', just to 'kinds' of plant  
 120 or animal. The concept of a biological species did not develop until the end of  
 121 17th century, and the above translation into English authorized by King James  
 122 is from the beginning of that century.

123 One recipe for the spontaneous generation of life is, "*take grains of wheat*  
 124 *and a sweaty shirt, place them under a box in a field, and leave for three weeks*".  
 125 The vapors of the shirt, together with the grains of wheat, would generate live  
 126 mice – which turned out to very similar to ordinary mice! Given the caterpillar  
 127 – chrysalis – butterfly example above, then couldn't human tissue make the

128 much simpler transformation into intestinal worms, or to liver flukes? Simi-  
129 larly, eggs of the barnacle goose could not be found and it was assumed that  
130 some invertebrates on the rocky shore turned into geese in the spring. The  
131 name barnacle is now associated with these marine invertebrates. In some other  
132 places it was assumed that melons turned into lambs; therefore (where meat  
133 was banned on religious grounds) eating lamb on Fridays was acceptable. In  
134 Paris, eating a particular bird as a fish was allowed during Lent, and the legend  
135 of the geese from barnacles was used to justify this. An early naturalist (John  
136 Ray, see Raven 1986) asked visitors to Paris to send him skins from the bird,  
137 and identified it as a sea-duck, the Scoter. Unfortunately (for eating meat on  
138 Friday) its reproduction was known.

139 It took centuries of careful work (Farley 1977, see also Oparin 1957) to move  
140 beyond European indigenous knowledge, to demonstrate that there was a  
141 continuity of like forms (species) over many generations. At this same time  
142 spontaneous generation was being questioned (and species were being con-  
143 sidered stable), early chemists were denying that chemical elements could be  
144 transmuted (lead could not be transmuted into gold, for example). Thus there  
145 was a similar movement in scientific thought:

146 denying continued spontaneous generation (non-living to living);  
147 denying transformation between chemical elements (non-living forms of  
148 matter); and  
149 denying transmutation between living forms (species therefore having a per-  
150 manence through time).

151 Thus we end up with the conclusion that early scientists discovered the  
152 problem of the origin of life, and that it was an important scientific advance to  
153 be able to reject continued spontaneous generation.

#### 154 **Statistical versus mechanistic (actualist) reasoning**

155 Having concluded that the ultimate origin of life is a scientific problem, it is  
156 time to consider two fundamental approaches, the statistical and the mecha-  
157 nistic (actualist). The late Fred Hoyle (physicist, broad thinker, and science-  
158 fiction writer) only considered the statistical approach and claimed that life was  
159 just too complex to arrive all at once; therefore life had to arise elsewhere in the  
160 universe and later be transported to earth – pangenesis. To him, it was just too  
161 improbable for even a single protein of only 100 amino acids to arise all at once  
162 and in the right order. If this probabilistic approach were the only one possible,  
163 then Hoyle's calculations show it is too hard (even given the Horowitz  
164 experiment on taking *Artemia* cysts down to absolute zero) for life to arise on  
165 Earth 'all at once'.

166 However, no researcher accepts this probabilistic approach; consider an  
167 analogy of a simple experiment that starts with an open beaker of bright blue  
168 solution of copper sulfate left on the bench. After coming back from vacation  
169 we find that the solution has disappeared, and that the beaker contains crystals

170 of copper sulfate. We could sit down and calculate the probability that at one  
171 instant in time all the water molecules evaporated, and that simultaneously all  
172 the copper and sulfate ions lined up in just the right configuration for the  
173 crystals. Yes, impossible.

174 Instead, we expect a process of millions of intermediate steps, each incre-  
175 mental step following known scientific principles. We could measure the  
176 chemical activity of water in the solution and of the atmosphere, and calculate  
177 the rate of evaporation through time. We could measure the solubility of  
178 copper sulfate in water at different temperatures; study the process of crystal  
179 formation in a range of compounds, etc. Similarly, with the origin of life we  
180 need to look for intermediate steps, and the principles behind the transitions;  
181 this is standard scientific approach to understanding events in the past was  
182 pioneered by James Hutton, Charles Lyell and Charles Darwin. Basically, we  
183 aim to explain the past by, in Lyell's phrase, 'causes now in operation'. We call  
184 this the *Principle of Continuity*.

185 Before leaving this analogy it is important to point out that the copper  
186 sulfate example is fully reversible. If we start with the crystals, and place a large  
187 volume of water adjacent to the beaker inside a closed container, water would  
188 diffuse back to the lower chemical potential of the crystals. They would slowly  
189 take up water, dissolve, and eventually give a similar solution to the one we  
190 started with. This is full reversibility, but is stronger than we require for a  
191 scientific explanation. Reversibility in the sense of physical chemistry just re-  
192 quires that a process can be broken down to large numbers of microscopic  
193 steps, each of which is reversible. This is the *Principle of Microscopic Revers-*  
194 *ibility* and it is also a standard scientific approach. It is this limited use of weak  
195 reversibility, or microscopic reversibility, that is equivalent to the 'continuity'  
196 used in the previous paragraph. In the early stages of the origin of life each step  
197 will be a normal chemical one; later it will probably be a genetic mutation.

## 198 **Introduction**

199 Having now got to the beginning of this analysis of the origin of life, the  
200 preintroduction establishes the problem as appropriate to scientific study,  
201 shows how the problem arose from careful observation and experiment, and  
202 shows that no simplistic probabilistic approach will help. At this point some  
203 form of definition of life would be useful, but because that is impossible to  
204 everyone's satisfaction, it is preferable to give the components that appear  
205 necessary. These are:

- 206 An energy source (an energy gradient),
- 207 Basic biochemistry (small molecules and reactions driven by the energy gra-  
208 dents),
- 209 Organization (membranes, compartmentation and separation from the  
210 external environment),

211 Self-reproducibility (genetic inheritability, information transfer, evolvability).

212 Clearly, not all four can appear simultaneously or we would be appealing  
213 to the 'statistical' (all at once) approach to the origin of life. We expect (as in  
214 any evolutionary system) a long series of intermediate forms, and therefore it is  
215 arbitrary to some extent which step we call 'living'. Here I will restrict the term  
216 'life' to a system that shows all four of the above features, and use 'prebiotic' to  
217 the intermediate (but essential) stages. Some of the controversial claims about  
218 the 'origin of life' are really about prebiotic, intermediate stages. Fine, they  
219 may be essential stages, but they still lack some components required for a full  
220 living and evolvable system.

221 You could protest that these four components focus on 'life as we know  
222 it' – couldn't there be other forms of life, say, based on silicon? Computers  
223 may eventually takeover. Certainly, slight variations on existing life are easy  
224 to imagine. At the simple end of the scale, a slightly modified form of life –  
225 yeast cells coding for 21 amino acids (rather than the 20 all current life  
226 uses) have already been made (Chin et al. 2003). Such possibilities are easy  
227 to envisage, and can probably be extended step by step until there are quite  
228 a few differences from standard life. But it is still hard to imagine and  
229 experiment with completely different forms of life and so I will consider  
230 both

231 general principles which apply to many possible forms of living systems, and  
232 our specific example of life, amino acids, ribonucleotides, polymerization,  
233 membranes, etc.

234 However, if we can solve the origin of our form of life then it will be easier to  
235 consider others.

236 Concurrently, I will usually give questions with a yes/no answer, simply to  
237 help direct a researcher to productive areas of research. We already have had  
238 two such questions; the life/Life alternatives, and the statistical versus mech-  
239 anistic (actualist) approach. One could protest that forcing binary choices is  
240 unrealistic for such a complicated process; but the approach is for convenience.  
241 We can always subdivide a search strategy into binary choices (even if it is not  
242 the optimal search path), and we can always reformulate a question. An  
243 example we consider later is whether life is more likely to start at high tem-  
244 peratures at the bottom of the ocean. After rejecting a 'hot start' at high  
245 temperatures (see Moulton et al. 2000) we realize that there are at least two  
246 questions about black smokers;

247 the optimal temperature for the early origin of life, and  
248 whether very high pressures are advantageous (rather than one atmosphere).

249 A third could be whether life could start in a dispersed aqueous phase, or  
250 whether it needs highly concentrated solutions at some stages. New questions  
251 can always be added. Thus binary choices are not limiting; we never consider  
252 all possible questions.

253 My approach here is a personal one in the sense that my aim is to give an  
254 analysis of critical questions, together with approaches that may allow their  
255 solution. The coverage includes both purely theoretical studies (such as the  
256 amount of information that can be transmitted for a given error rate) as well as  
257 the better known experimental results. This combination of theoretical and  
258 empirical studies is unfortunately not common. No doubt experimentalists often  
259 think theoreticians ignore important experimental information. Similarly, the-  
260 oreticians find it hard to believe that experimentalists are so slow in following up  
261 their brilliant ideas. For example, the early work of Eigen and Schuster (1977)  
262 indicated to many theoreticians that studying RNA offered the only possibility  
263 for real progress. This was strongly reinforced when Reaney (1982) showed  
264 that the Eigen/Schuster concepts explained many features of RNA viruses.  
265 However, it seemed a very long time before the experimental work on RNA  
266 systems started. It is important to increase dialogue between the two groups.

267 My aim here is not to cite every last reference, but to identify productive  
268 areas of research and to look for others where current assumptions may be  
269 hindering progress. There are excellent reviews of different aspects of origin of  
270 life studies, and they will give more detailed sources. The origin of the earth  
271 and the solar system is well covered in the recent book by Conway Morris  
272 (2003) and is not part of this overview. Similarly, the discovery of water (ice) on  
273 Mars (Bibring et al. 2004) opens possibilities for the future, but until we have  
274 data for the extraterrestrial origin of life, it is outside our scope.

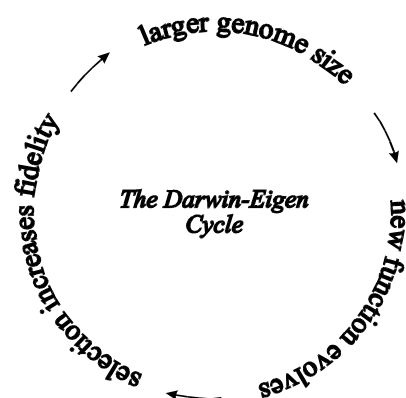
## 275 **Principles or guidelines**

276 Physical and chemical principles, including kinetics, catalysis, thermodynamics  
277 and quantum mechanics (including energy sources and entropy) are taken for  
278 granted, as is the parsimony principle of seeking the simplest theories that are  
279 effective. Similarly, natural selection is also assumed, though some of its con-  
280 sequences are given in more detail below. It is sufficient to say that natural  
281 selection is the consequence of the combined effects of genetic and ecological/  
282 populational processes. Ecology includes the potential for exponential increase  
283 of numbers and competition for limited resources. Genetics gives heritable  
284 variability, some of which may increase the probability of survival and  
285 reproduction. The fundamental question, as discussed in Penny and Phillips  
286 (2004) in a different context, is whether the processes we can study in the  
287 present are sufficient to explain the origin of life. Given the above principles,  
288 the points to emphasize here are as follows.

### 289 *Immediacy/current utility*

290 There is no foresight or planning for future utility, this is basic to darwinian  
291 evolution. One of the strongest limitations of such a mechanism is that it can't





*Figure 1.* The Darwin–Eigen cycle. A positive feedback loop where an increase in replication accuracy allows longer sequences to be retained (that is, increases the Eigen-limit) which in turn allows more genes to be coded, allowing selection for a further increase in replication accuracy.

### 327 *Universality through time*

328 Principles were as relevant in the past as they are in the present; indeed some,  
 329 such as the Eigen limit on genome size, were probably more critical in the past  
 330 when error rates of replication were higher. Other problems such as keeping  
 331 good combinations of genes together (avoiding random gene loss) and pre-  
 332 venting parasitic genes entering the system are acute in early systems. It is often  
 333 assumed that the advantages of recombination of genes through sexual  
 334 reproduction was a new invention in eukaryotes, but linking advantageous  
 335 genes together (whilst still allowing recombination) would have had major  
 336 advantages as far back as the RNA-world (see later).

### 337 *No reverse takeover by RNA*

338 One principle we have used (Jeffares et al. 1998) is based on the observation  
 339 that proteins are several orders of magnitude faster, and more specific in their  
 340 reactions, than RNA catalysis. For example, RNA-catalyzed reactions may  
 341 have turnover times of several minutes (see Table 1, Jeffares et al. 1998)  
 342 whereas protein-catalyzed reactions are often 10,000 to 100,000 times faster.  
 343 Thus we do not expect RNA to take over a catalytic role that proteins are  
 344 already doing. This allows a direction of change for some early events (see  
 345 later). Under this principle, RNA may develop new functions, including reg-  
 346 ulatory functions, amongst modern organisms; it is just that it does not  
 347 takeover catalytic functions from proteins.

348 *Autocatalytic cycles.*

349 A relatively new mathematical analysis (Hordijk and Steel 2004; Mossel and  
350 Steel, in press) shows that autocatalytic cycles can occur with reasonable  
351 frequency in a mixture of chemicals that are potential substrates and/or  
352 catalysts. In an overly simplistic cycle, molecule A could catalyze the for-  
353 mation of B, B of C, and C of A. In reality many more reactions and side  
354 reactions could occur. In biochemistry the focus has been on macromolecules  
355 (especially proteins) catalyzing reactions. However, weak catalysis is a  
356 property of many small molecules and metallic ions, albeit with much lower  
357 rates. For example, we think of the break down of hydrogen peroxide ( $H_2O_2$ )  
358 to water and oxygen being carried out by the protein enzyme catalase.  
359 However, catalase has a heme molecule as a coenzyme (cofactor) that is  
360 involved in this chemical reaction, and the heme molecule by itself (without  
361 the protein) is a weak catalyst of the reaction. Again, the metal at the active  
362 center of heme,  $Fe^{2+}$ , is also a catalyst, even though even weaker than heme.  
363 Thus small molecules and ions are also catalysts. Autocatalytic cycles work in  
364 mathematical equations (Hordijk and Steel 2004) and are an important fea-  
365 ture for future work.

366 These principles are all important in the search for a good explanation for  
367 the origin of life, and equally perhaps for rejecting others that rely on un-  
368 testable and unlikely events. For a good explanation we require known reac-  
369 tions and mechanisms, a plausible series of intermediates, and ultimately  
370 testable predictions. In geology, the approach of explaining the past in terms of  
371 causes or mechanisms still available we call actualism (Camardi 1999). With  
372 those principles in mind it is time to consider some of the many steps from an  
373 early earth to living systems.

374 **Chemical and biological starting points**

375 It is standard to divide origin of life studies into the forwards and backwards  
376 directions.

377 *Bottom up (chemical) approach*

378 The forward (chemical, or bottom up) approach starts with the chemicals and  
379 conditions existing on the early earth (or elsewhere if you are of that persua-  
380 sion). It studies the small molecules that would form, and how they might  
381 polymerize into larger molecules. This approach goes from the simpler to the  
382 more complex, towards a simple living system. It is more the domain of  
383 chemists and geophysicists. Topics include the following:

384 Conditions during the early stages of formation of a planetary system,

- 385 Detecting small organic molecules in asteroids or in gas clouds in space –
- 386 towards exobiology,
- 387 Origin of small organic molecules under possible prebiotic conditions,
- 388 Formation of order (phase separation) and membranes,
- 389 Polymerization of nucleotides and/or amino acids,
- 390 A preRNA-world (which could have been quite extensive).

391 *Top down (biological) approach*

392 The backwards (biological, or top down) starts with life as we know it and  
 393 simplifies it stepwise. What is the simplest system that could have evolved  
 394 into 'life as we know it', using known mechanisms? For example, hemoglo-  
 395 bins are not useful before high oxygen concentrations occurred in the  
 396 atmosphere, so we delete this protein from our proposed early cell. This is  
 397 still within our Principle of Continuity because we know from gene dupli-  
 398 cation and the subsequent divergence of function of the copies that we could  
 399 recover hemoglobin from other proteins. This top-down approach is the  
 400 territory of biologists and paleontologists. Subject areas for the working-  
 401 backwards approach include:

- 402 Geology and the earliest fossils (though are the very earliest fossils even
- 403 DNA-based organisms),
- 404 The Last Universal Common Ancestor (LUCA),
- 405 Establishing a DNA world, the origin of DNA with its takeover of infor-
- 406 mation storage from RNA (except for RNA viruses),
- 407 A ribonucleoprotein (RNP) world (protein takeover of most catalysis from
- 408 RNA),
- 409 An RNA-world (omitting DNA and proteins) with RNA catalysis and
- 410 information storage,
- 411 RNA viruses as exemplars of the RNA-world. RNA viruses have both high
- 412 rate of replication error (about a million-fold higher than DNA organisms)
- 413 and consequently have short and/or fragmented genomes. Reaney (1982)
- 414 initially pointed out how RNA viruses illustrated principles such as the Eigen
- 415 limit. However, they will not be discussed further, it is sufficient to say that the
- 416 properties of RNA viruses are fully in agreement with theoretical predictions.
- 417 Having given the forwards and backwards approaches, and for reasons that
- 418 will become apparent, I will use neither – but jump straight to a potential mid-
- 419 point, the proposed RNA-world. This is my Theme one, and illustrates the
- 420 wide range of phenomena explained by the RNA-world model. Theme two
- 421 then outlines a plausible set of stages to get from an RNA-world, via proteins,
- 422 to DNA-based organisms, and thus to modern living forms. This covers the
- 423 backward direction from extant organisms. Theme three considers alternatives
- 424 (or variations) to RNA – how small or large is the target for chemical simu-
- 425 lations (the forwards reaction – Theme four). Without an RNA-world target it

426 is not at all obvious how to use to choose between competing ideas on the early  
427 stages in the origin of life. But it is already a complex target.

#### 428 **Theme 1 – The RNA-world**

429 *Which came first – the chicken or the egg, protein or DNA?*

430 At one time, the separation of function between DNA and proteins was a  
431 major stumbling block in the search for the simplest living system. Consider a  
432 DNA molecule. It carries information that can be replicated and passed on to  
433 descendants, but does not catalyze any metabolic reactions in the cell. It is a  
434 boring molecule from the viewpoint of catalysis. It is like a book in a library –  
435 it may contain information but it does not do anything; someone has to read  
436 the book before its information is useful.

437 On the other hand, proteins catalyze thousands of reactions with great speed  
438 and high precision. But proteins apparently do not directly reproduce the  
439 information in their own sequences; any change to a protein's sequence is not  
440 inherited. This left the apparent problem of 'which came first' – DNA that  
441 carried information but does not 'do' catalysis in modern cells, or proteins that  
442 carried out reactions but could not reproduce. This gave a classic, 'which came  
443 first, the chicken or the egg?' problem. Avoiding this problem has a delightfully  
444 simple solution.

445 *The RNA-world: no protein, no DNA – no chicken, no egg.*

446 The RNA-world hypothesis is conceptually simple: at one stage during the  
447 origin of life RNA both stored information and was the main macromolecule  
448 carrying out catalysis. In other words, in the origin of life

449 RNA preceded (genetically encoded) proteins, and  
450 RNA preceded DNA.

451 The theory only considers the genetic (information) aspects of biology. It  
452 does not directly consider energy sources, the status of proto-metabolism, nor  
453 cellular organization. Amino acids are very likely to have been present and may  
454 have formed short peptides. However, their sequences would not have been  
455 genetically-coded in the modern sense. I will restrict the term 'protein' to cases  
456 where the amino acid sequences are heritable and (after Fox and Dose 1972)  
457 use 'protenoid' for peptides whose sequences are not inheritable (but still may  
458 have been very important). Returning to RNA, the following list gives the main  
459 roles for RNA (and its metabolic components). The first ones were discovered  
460 early, and the Introduction of Gesteland and Atkins (1993) covers these early  
461 discoveries. However, new roles for RNA keep being discovered in what is a

462 very active field. These functions (Jeffares et al. 1998; Meli et al. 2001; Joyce  
463 2002) include:

464 RNA (like DNA) carries genetic information (as messenger RNA [mRNA] or  
465 as the genome in RNA viruses);

466 RNA has both catalytic and structural roles in ribosomes (rRNA) - it is both  
467 the core structure of the ribosome, and catalyses the polymerization of  
468 amino acids into proteins (Steitz and Moore 2003);

469 RNA (as tRNA) translates between the mRNA code [triplets] and amino  
470 acids;

471 Ribonucleotides (building blocks for RNA biosynthesis) are precursors for  
472 deoxy-ribonucleotides (building blocks for DNA biosynthesis) – thus ribo-  
473 nucleotides probably existed first. Indeed it is a difficult reaction, requiring  
474 complex proteins, to get from ribose to deoxyribose nucleotides (Poole et al.  
475 2001);

476 RNA is a primer for DNA synthesis (when a new section of DNA is syn-  
477 thesized, an RNA primer (which is later replaced by DNA) is required to  
478 initiate DNA synthesis – in this sense, DNA cannot be replicated without  
479 RNA);

480 RNA (as ribozymes, from ‘ribo’ and ‘enzyme’) has many catalytic roles,  
481 especially in processing RNA itself (Doudna and Cech 2002). There is a  
482 cascade of RNA processing effects; snRNA (small nuclear RNAs) act on  
483 snoRNA (small nucleolar RNA) which acts on rRNA (ribosomal RNA) –  
484 RNA processing a second RNA which processes a third RNA;

485 Most organic coenzymes [cofactors] of protein enzymes have ribonucleotide  
486 components (e.g. FAD, NAD, NADP, pterins and coenzyme A) and these  
487 coenzymes are essential for proteins carrying out the chemical reactions. For  
488 example, FAD is an abbreviation for flavin adenine dinucleotide. It has a  
489 classic dimer ribonucleotide structure; supporting the suggestion that these  
490 essential coenzymes predate protein enzymes;

491 RNA has many regulatory roles within cells [such as RNA interference  
492 (RNAi, Novina and Sharp 2004) and ribo-switches (Winkler et al. 2004)]  
493 where RNA molecules detect and respond to the presence of specific mole-  
494 cules in the cell;

495 The RNA-world hypothesis largely avoids the problem that amino acids exist  
496 in two mirror images (D and L) that would inhibit growth of consistent 3-D  
497 structures in pre-biological molecules. (It is likely that the L-form of ribose  
498 determines the D-form of amino acids during protein synthesis (Tamura and  
499 Schimmel 2004), though this still leaves the problem of isomers of ribose.)

500 Eukaryotic genes have an exon/intron structure where exons (usually) code  
501 for proteins and introns are excised and (usually) discarded. A very complex  
502 RNA/protein spliceosomal machinery exists to remove introns and the RNA-  
503 world accounts for this complexity. (It is difficult to see advantages in  
504 developing introns and the splicing apparatus if they were not already present  
505 in the earlier RNA-world; this is the introns-first theory, Poole et al. 1998. At

506 a minimum, introns indicate how an RNA-world could be organized even if  
507 they were lost and then later regained in eukaryotes.)

508 The synthesis of protein is carried out by the ribosome, which is very much  
509 an RNA machine – all the central parts that carry out the synthetic reactions  
510 are RNA (Steitz and Moore 2003). Conversely, proteins carry out DNA syn-  
511 thesis, including the difficult conversion of RNA precursors (ribonucleotides)  
512 to DNA precursors (deoxyribonucleotides). This reduction of ribose to  
513 deoxyribose involves a free-radical reaction (Poole et al. 2001) and is well  
514 beyond the catalytic repertoire of RNA as currently known. Thus, in an  
515 important sense, RNA makes proteins, and proteins make DNA. Although not  
516 central to the RNA-world hypothesis it is now assumed that protein preceded  
517 DNA, giving the sequence

RNA → protein → DNA.

519 It is this version that I use here. It is very important to note that there is no  
520 claim that RNA was the ‘first’ self-replicating molecule; just that genetically  
521 RNA preceded both protein and DNA. Indeed, possible precursors of RNA  
522 are an important topic that comes later. Similarly, there must have been a  
523 complex cell and metabolism by the time of the RNA-world; these aspects are  
524 discussed later.

525 There are many functions of RNA in modern organisms, which ones might  
526 date back to an RNA-world? For this question we use the principle outlined  
527 earlier that, because protein is more effective in catalysis than RNA (in terms of  
528 both rates and specificity), we do not expect RNA to ‘take back’ a role that  
529 proteins were doing. Certainly, RNA functions can increase in a cell by normal  
530 evolutionary processes such as gene duplication, followed by divergence of one  
531 of the copies. Our restriction is just that RNA would not takeover a catalytic  
532 role from proteins. To identify relics from the RNA-world we (Jeffares et al.  
533 1998) looked for functions that were central to metabolism (and therefore  
534 likely to be old), were widespread in different organisms, and were catalytic.  
535 The list (Jeffares et al. 1998) was quite impressive, and growing.

#### 536 *In vitro evolution of RNA catalysis*

537 The simultaneous discovery (by Altman and Cech, see Gesteland and Atkins  
538 1993) that RNA, even in the absence of proteins, catalyzed reactions was quite  
539 dramatic in that it was unexpected by the general biochemical community.  
540 Nevertheless, many theoretical biologists had favored RNA as an early func-  
541 tional molecule – even in the absence of direct biochemical evidence for its  
542 catalytic activity. It was not that biochemists were against RNA being  
543 important in the early origin of life; biochemists studied ‘real’ reactions that  
544 occurred in the present, not imaginary reactions that might have existed in the  
545 past. However, once reactions catalyzed by RNA were discovered, the way was

546 open to evolve ribozymes artificially through repeated rounds of synthesizing  
547 RNA (with partial randomization) and selection. This is evolution *in vitro*.

548 The process of *in vitro* evolution is conceptually simple.

549 Synthesize billions of variants of a nucleic acid sequence (this is straightfor-  
550 ward by using mixtures of the four nucleotides for at least parts of the  
551 synthesized sequence).

552 Select any variants that may have even a weak function. Passing the syn-  
553 thesized molecules through an appropriate column is a frequent method;  
554 those molecules retained on the column can then be eluted later by a more  
555 concentrated solution).

556 Copy the selected RNA, often under conditions where the polymerase is  
557 'error-prone'. This amplifies the molecules selected in the second step, but also  
558 can generate new variants differing in only a small number of mutations.

559 The second and third steps are repeated until RNA molecules with the de-  
560 sired activity are found. Calling these 'evolved ribozymes' distinguishes them  
561 from those occurring naturally. There are many variants on the basic method,  
562 but all have the cycle of starting with large numbers of variants, selection,  
563 copying, and repeating the cycle of selection and synthesis. Because of its  
564 greater chemical stability, most start with DNA, copy it to RNA, carry out  
565 selection on the RNA molecules, and then use a reverse transcriptase reaction  
566 to copy the selected RNA back to DNA for further rounds of replication and  
567 selection.

568 Only two applications of *in vitro* experiments are mentioned here, evolving  
569 an RNA polymerase, and demonstrating that protein coenzymes can function  
570 with ribozymes. For the first application, it is essential for any RNA-world  
571 theory that RNA can be an RNA-polymerase, and there is reasonable success  
572 in this respect (Lawrence and Bartel 2003). We will return to this reaction later  
573 under the origin of the ribosome with a suggestion how to improve the existing  
574 RNA-based RNA-polymerases. Turning to coenzymes, Jadhav and Yarus  
575 (2002) summarize a large number of evolved ribozymes that use (ribonucleo-  
576 tide) coenzymes (FAD, NAD, etc.), which are now only associated with pro-  
577 tein enzymes. Overall, the results are a real success for the RNA-world theory;  
578 RNA can catalyze many chemical reactions.

579 Initially it was assumed that RNA was inherently better than DNA as a  
580 catalytic macromolecule – and that explained why there were no naturally  
581 occurring deoxy-ribozymes. 'It was the catalytic role of the additional hydroxyl  
582 (-OH) group on ribose that made RNA such a good catalyst', was a common  
583 argument. This was a typical *post hoc* explanation in biology; find something in  
584 nature and dream up an explanation. In this case the 'explanation' is wrong as  
585 a generalization. Artificial DNA ribozymes can be created, and are at least as  
586 effective as their RNA counterparts – and are more stable. For example,  
587 Chinnapen and Sen (2004) give an important case of a deoxy-ribozyme that, in  
588 the presence of light, repairs thymine dimers in DNA. Thus the existence of an  
589 extensive set of naturally occurring RNA (not DNA) ribozymes is important in  
590 itself and is evidence for their antiquity, relics of the RNA-world. Nowadays,

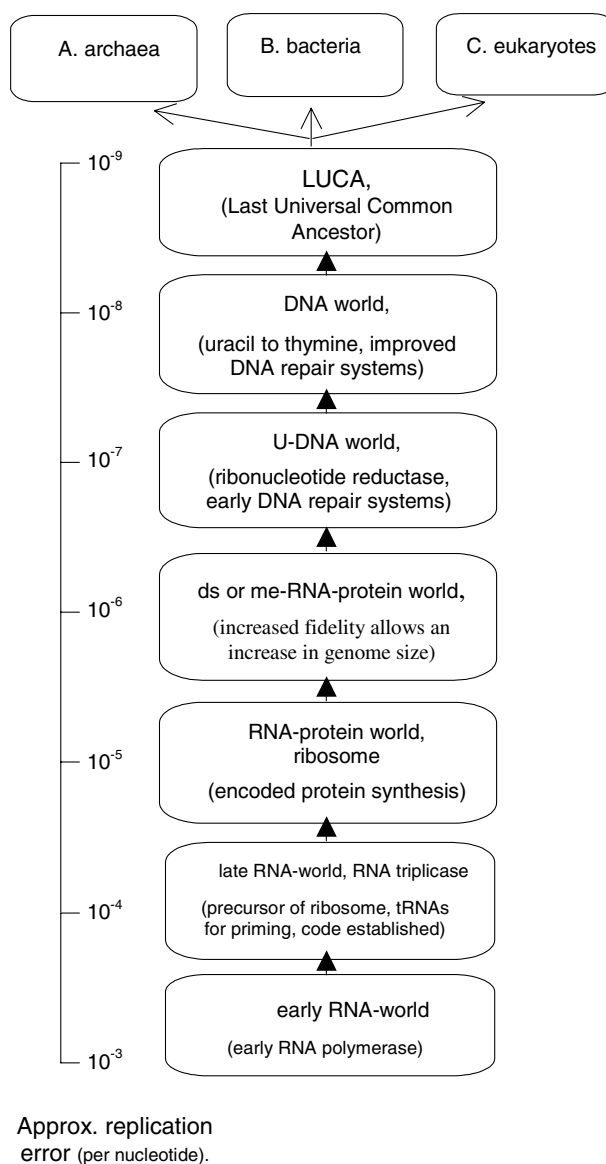
591 starting from modern biochemical knowledge, and given the higher stability of  
592 DNA, we might 'design' at least some ribozymes from DNA. A recent review  
593 on *in vitro* evolution of both RNA and DNA is Joyce (2004).

594 The RNA-world hypothesis has been an outstanding success. It started with  
595 rather vague suggestions in the 1960s and 70s that RNA must have been  
596 important early (see Gesteland and Atkins 1993, Chapter 1). This was followed  
597 by the quantitative work of Eigen and Schuster (summarized in Eigen 1992)  
598 who established some fundamental principles. In the style of Sherlock Holmes,  
599 when everything else is impossible, we have to accept the only alternative left –  
600 even if it appears unlikely. In this context, the discovery of RNA catalysis was  
601 almost necessary! In retrospect there was a conceptual problem – the early  
602 focus was on DNA as the information storage molecule and on proteins as the  
603 catalysts. RNA was considered just an intermediate. All that has changed and  
604 the slogan is now 'RNA can do [almost] anything'. But how do we get to an  
605 RNA-world, and how do we get from an RNA-world to the protein and DNA  
606 worlds?

## 607 **Theme 2 – From the RNA-world to proteins and DNA**

608 A strength of the RNA-world hypothesis is that a plausible step-by-step model  
609 can be developed, first for the origin of the genetic code, which then extends the  
610 model to the evolution of protein synthesis, and later the evolution of DNA.  
611 These steps allow the take-over of most catalysis by proteins, and most  
612 information storage by DNA. On our model, the basic driving force is the  
613 positive feedback loop of the Darwin–Eigen cycle (Figure 1). There are suc-  
614 cessive improvements in the accuracy of replication followed by increased  
615 information storage, allowing increased replication fidelity, and so on  
616 (Figure 2). The model has many intermediate steps, each following our prin-  
617 ciples of continuity, weak reversibility, and immediate utility.

618 From our earlier observation that 'RNA makes protein, and protein makes  
619 DNA' the reader will predict that the origin of protein synthesis must be the  
620 first major step from an RNA-world. This leads to an RNP-world (RiboNu-  
621 cleoProtein), a combination of RNA and protein. Proteins have a superior  
622 catalytic ability to RNA in both rate and specificity (both substrates and  
623 products). For example, RNA-catalyzed reactions may have turnover times of  
624 several minutes (see Table 1, Jeffares et al. 1998) whereas protein-catalyzed  
625 reactions are often 10,000 to 100,000 times faster (and more specific). This  
626 comparison is a little unfair in that proteins increasingly took over catalysis  
627 involving smaller molecules and so were less limited by the diffusion times of  
628 the reactants onto the macromolecular catalyst (Albery and Knowles 1976).  
629 (Neglecting solvation affects, the average rate of diffusion in solution is in-  
630 versely proportional to the square root of the molecular weight.) However, this  
631 protein takeover reflects the advantage of improved catalysis when catalysis



*Figure 2.* The Darwin–Eigen cycle in practice. A series of steps allowing a succession of increases in replication accuracy followed by an increase in the size of the genome. Every time the accuracy of replication is increased, it allows in principle in increase in the amount of information coded. This, in turn, allows for the selection of an increase in accuracy, which allows more coding, which allows selection for more information, and so on. (See the Darwin–Eigen cycle, Figure 1.)

632 (rather than diffusion) is limiting. Nevertheless, RNA would have retained the  
 633 coding (information storage) role in an RNP-world. However, the major  
 634 difficulty is getting from an RNA-world to encoded protein synthesis.

635 *Origin of the ribosome, origin of the triplet amino acid code*

636 The main problem is that the origin of ribosome and of the triplet code for  
637 amino acids cannot have arisen 'for' protein synthesis – the usual explanation.  
638 The ribosome is a huge macromolecular complex and there are many steps  
639 leading up to the synthesis of the peptide bond that joins amino acids. There is  
640 no way that a ribosome could have evolved *de novo* in a single step and meet  
641 our guidelines of continuity (no miracles). It is impossible on an incremental  
642 model that a very complex structure could evolve 'for' something that does not  
643 yet exist.

644 A standard evolutionary explanation is that a new feature (in this case, the  
645 triplet code and the ribosome) evolved for a different purpose, and was later  
646 recruited or co-opted to a new role, in this case into protein synthesis. The  
647 classic examples are duplication of a single gene with one copy being recruited  
648 into a new function, one copy retaining the original role. A typical example is a  
649 gene for an early hemoglobin molecule being duplicated, one copy becoming  
650  $\alpha$ -hemoglobin, the other  $\beta$ -hemoglobin. Could something similar have hap-  
651 pened with the ribosome? We must consider a prior function for a proto-  
652 ribosome, a function that allowed it to be co-opted into protein synthesis. I will  
653 discuss the origin of the ribosome in some detail to use it as an example of  
654 developing a testable evolutionary hypothesis for a very complex process.

655 At first sight there are two major problems; there is no obvious relic in  
656 modern cells of an RNA-based RNA-replicase, nor any obvious function for a  
657 proto-ribosome. However, there is a simple and powerful solution to both  
658 problems; namely, the proto-ribosome was initially involved in RNA replica-  
659 tion, and later was recruited into protein synthesis (Poole et al. 1998; 1999; see  
660 also Gordon 1995). Indeed, there must have been a high-accuracy RNA  
661 polymerase in the RNA-world. Because of the Eigen-limit (around one error  
662 per replication) RNA had to be copied with a high fidelity – at least as accurate  
663 as modern protein-based RNA polymerases. Such a proto-ribosome recruited  
664 into protein synthesis could be a large RNA complex that included the  
665 following (see Table 1 and Figure 2 of Poole et al. 1998).

666 A total length of ribosomal RNA around 4500 bp. This is similar to current  
667 ribosomes – once proteins were available there appears little advantage in  
668 increasing the length of the RNAs.

669 The proto-ribosome recognizes and attaches to a single stranded RNA  
670 (ssRNA) which is being replicated (mRNA is the equivalent of this ssRNA in  
671 the modern ribosome).

672 tRNA-precursors that donate triplets of ribonucleotides to replicate the  
673 ssRNA. This reverses the role of the anticodon and the amino acid in modern  
674 tRNAs. Currently, tRNAs are donors of amino acids identified by the triplet  
675 (anticodon) on the tRNA – the triplet is kept, the amino acid is added to the  
676 protein. On our protoribosome model, the triplet was added to the growing  
677 RNA chain, and the amino acid on the tRNA (which aided in recognition and  
678 specificity) was retained.

679 A ratchet mechanism that moves the ssRNA being replicated through the  
680 proto-ribosome in steps of three nucleotides (this explains why the amino acid  
681 code is three nucleotides long, see later).

682 Adding ribonucleotides three at a time is expected to be more accurate in  
683 RNA polymerization than adding one at a time, I will come back to this later.  
684 Our model is that the proto-ribosome was an RNA-polymerase ribozyme that  
685 added triplets of nucleotides to the growing RNA molecule (Poole et al. 1999).  
686 We call this a 'triplicase'. To a biochemist, the triplicase has properties of both  
687 a ligase and a polymerase. A ligase because it joins (ligates) two RNA mole-  
688 cules; the growing chain plus the triplet. A polymerase because it repeats the  
689 operation sequentially along the new RNA strand (a ligase is usually involved  
690 in just a single reaction on a particular molecule).

691 Why triplets? Because it gives the length of the code, is a superficial answer.  
692 The origin of the triplet code is a similar problem to the origin of the ribosome.  
693 There is just no way under a Darwinian model that a complex code could have  
694 evolved 'for' something that did not exist – namely encoded protein synthesis.  
695 An alternative and frequent answer is evolutionarily obscure – the code is a  
696 triplet 'because' it allows 20 amino acids to be coded. This requires long-range  
697 planning and forethought! Thus the origin of the code must be broken into two  
698 questions.

699 Why three nucleotides?

700 How did our existing triplet code for amino acids arise?

701 In the above model, only the 'three nucleotide' question is discussed, not the  
702 origin of the code we observe today. How plausible is this model for the origin  
703 of the ribosome? Can it lead to predictions?

704 *Could the ribosome have evolved from an ancient RNA-replicase?*

705 When thinking in evolutionary terms we are used to considering time-scales  
706 from hundreds to millions of years. However, when treating issues of the  
707 accuracy of replication our time scale is that of chemical reactions, and events  
708 in millionths of a second are important. The issue is the extremely short time  
709 over which, for example, a free cytosine (C) ribonucleotide will H-bond with a  
710 guanine (G) in the RNA molecule being copied, to give a C≡G pairing. Protein  
711 polymerases can accurately incorporate single nucleotides. Indeed, Piccirilli  
712 et al. (1990) show that novel base pairs can be incorporated into both RNA  
713 and DNA (see later) so it appears that the information from complementary  
714 base pairing (C≡G, A = U) is sufficient for RNA and DNA replication when  
715 proteins are doing the catalysis. However, it is expected that the much longer  
716 reaction times of ribozymes (Jeffares et al. 1998, Table 1) would tend to allow  
717 dissociation of the template and lose the incoming (free) nucleotide before the  
718 reaction was complete. This is expected to lead to a higher error rate of rep-  
719 lication (and possibly leading to a requirement for pre-tRNAs that carry the  
720 nucleotide). This conclusion is consistent with the lower accuracy of the present

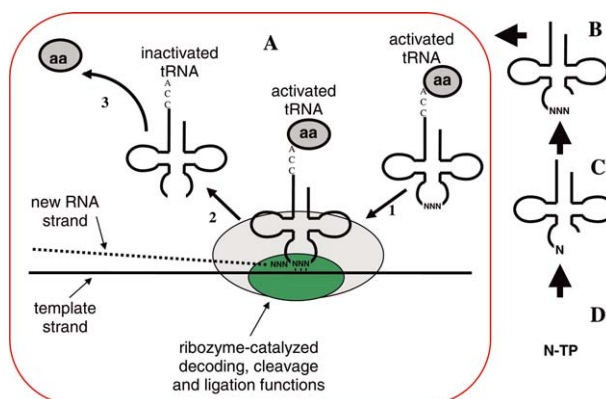


Figure 3. Model for the origin of the ribosome with an RNA triplicase that adds three nucleotides at a time to copy a RNA template. (a) is a very late stage where the triplet code is established and tRNAs are charged with amino acids and which enhances the accuracy of triplet addition to the growing RNA chain. The three main steps are shown with charged tRNAs, recognition of the RNA by binding of the anticodon, and release of the tRNA following ligation of the anticodon to the growing RNA chain (probably with release of the amino acid). Then we work backwards to earlier stages. (b) uses a pre-tRNA to add three nucleotides at a time, but without an amino acid to enhance specificity. (c) is an earlier stage with a pre-tRNA donor that enhances specificity, but adds only a single nucleotide at a time. (D) The earliest stage where nucleotides are added singly and specificity depends solely on nucleotide pairing. Clearly, the order of some events (one or three nucleotides, with or without pre-tRNA) could be reversed.

721 day RNA-based RNA polymerases formed by *in vitro* evolution (Lawrence and  
722 Bartel 2003).

723 Thus an advantage of trinucleotide over single nucleotide addition is that  
724 trinucleotides H-bond longer to the RNA template, giving our hypothetical  
725 replicase (Figure 3) more time for polymerization. For example, we expect  
726 the trinucleotide AGU to base pair longer to its complement UCT, than  
727 simply a U opposite a T. Increasing the length of an oligonucleotide increases  
728 the stability of base pairing, which can become unexpectedly stable in the  
729 interaction of two complementary triplets of tRNAs (Grosjean et al. 1976).  
730 However, the number of possible oligonucleotides also increases exponen-  
731 tially; there are 16 pairs of nucleotides (AA, AC, AG, etc.) but 64 possible  
732 triples (AAA, AAC, etc.) – for each additional nucleotide there are four times  
733 as many potential substrates. In principle, oligonucleotides larger than triplets  
734 are possible, but only triplets are consistent with the origin of the triplet code  
735 for protein synthesis. This is really a description of the problem, and does not  
736 explain how the length of the code (a triplet) could have arisen. This is  
737 discussed again later.

738 Although the discussion has been on co-opting a proto-ribosome into  
739 protein synthesis, the model for the evolution of the proto-ribosome itself is  
740 also incremental, and driven by the Darwin–Eigen cycle. The early protori-  
741 bosome could evolve through a number of modules, there is no need to

742 invoke massive new complexes of interacting catalytic RNA arising in one  
743 step. What is now the 16S rRNA may have been the earliest component as an  
744 RNA-polymerase; the 23S rRNA (now the peptidyl transferase) a later  
745 addition that initially formed the ratchet mechanism (Poole et al. 1999). A  
746 model with amino acid tags, initially perhaps positively charged amino acids,  
747 could improve fidelity by aiding in recognition of nucleotides at the anticon-  
748 don end, or by stabilising the interaction between the negatively charged  
749 RNAs. This use of amino acid tags bound to the tRNA, with trinucleotides  
750 being added to the growing RNA chain means that a relationship between  
751 codon and amino acid is already forged. The full genetic code need not have  
752 arisen at this point but a triplet code is at least partially established well  
753 before protein synthesis (Figure 3).

754 *Experimental testing of the ribosome model, actual and potential*

755 The first test of a model is its logical consistency and its plausibility based  
756 on known mechanisms. At this level, I think the above hypothesis for the  
757 origins of protein synthesis is the only one that even comes close to meeting  
758 these criteria – at the moment there are no other scientific models for the  
759 origin of the ribosome. There is one new experiment that supports our  
760 model, and it is critical. This is the report by Fredrick and Noller (2003)  
761 that the ribosomal RNA itself carries out the ratchet mechanism of moving  
762 the mRNA along one triplet, with concordant movement of the tRNAs –  
763 the movement does not require proteins. This really is a stunning finding; it  
764 is arguably the most important finding about the origin of the ribosome this  
765 new millennium! It supports the idea that the rRNA/ss(m)RNA/tRNA  
766 interactions are ancient, from before proteins. The RNA is the core struc-  
767 ture of the ribosome (and presumably of the proto-ribosome). It is both the  
768 catalyst for the formation of the aminoacyl bond between two amino acids,  
769 and carries out the major movements of tRNA and mRNA. Recent work  
770 (Hoang et al. 2004) extends the conclusion still further in that proteins close  
771 to the P-site of the ribosome (which is critical for joining amino acids) can  
772 be mutated so that the protein no longer touches the RNA; and the ribo-  
773 some still functions.

774 The Fredrick and Noller (2003) result, though critical, was not a planned test  
775 of the model. There are good possibilities for tests in the future; the main  
776 foreseeable one is to extend (by standard RNA *in vitro* evolution methods) the  
777 ability of RNA-based RNA-polymerases. Consider the following, in a series of  
778 *in vitro* evolution experiments Lawrence and Bartel (2003) report a ribozyme  
779 RNA-polymerase. In the initial report (Ekland and Bartel 1996) the ribozyme  
780 added the first ribonucleotide fairly quickly, then the second, and finally a third  
781 much more slowly. It took forever to add any more. A similar conclusion  
782 about three nucleotides comes from the experiments by McGinness et al.  
783 (2002). They evolved a ribozyme that adds two nucleotides to a growing RNA

784 chain, then ligates a short RNA chain. Basically, this ribozyme is carrying out  
785 three successive reactions from a single attachment between ribozyme and  
786 ssRNA.

787 For both the Bartel and McGinness results, it appears that their ribo-  
788 zymes lack full 'processivity', they lack the ability to keep moving along the  
789 ssRNA being replicated. Our interpretation is that the replicases are only  
790 flexible enough to add three nucleotides at their active site. After three  
791 nucleotides, a ribozyme could detach and then reattach three nucleotides  
792 further along the ssRNA – a tricky operation at the best of times. Much  
793 better would be to have a ratchet mechanism that moved the ssRNA three  
794 nucleotides through the replicase. This, the 3-step ratchet mechanism, would  
795 be the origin of the RNA triplicase, the proto-ribosome, and the length of  
796 the triplet code.

797 The critical experiment is to aim to do just that, look for a 3-step ratchet.  
798 Start with the existing single-chain RNA polymerases from *in vitro* evolution  
799 experiments (Lawrence and Bartel 2003) and evolve, by standard *in vitro* RNA  
800 evolution techniques, a second RNA molecule that interacts with the first.  
801 Then search for variants that allow RNA synthesis to continue at an unin-  
802 terrupted rate. In principle, it doesn't matter if the mRNA is moved in steps of  
803 three, just that if the moves are in steps of three then this is a prototype for a  
804 proto-ribosome (see Figure 3). Initially in such a system ribonucleotides are  
805 still added singly, but there would be later advantages for increasing replication  
806 accuracy by adding triples of nucleotides (Poole et al. 1999). Basically we are  
807 back to the Darwin–Eigen cycle, where increased accuracy allows longer  
808 nucleotide sequences to be maintained, and for RNA the relatively low repli-  
809 cation accuracy is a major limit to information storage. An RNA-world  
810 absolutely needs a high fidelity RNA-polymerase. If the above experiment was  
811 successful – evolving a second RNA molecule that allowed continued RNA  
812 replication – it would be my candidate for the top RNA-world experiment in  
813 the next decade.

814 The RNA triplicase theory is attractive in that it gives a high fidelity repli-  
815 case/polymerase in the RNA-world, an origin for a triplet code, and an origin  
816 for the ribosome with its RNA processing cascade. It is possible in principle to  
817 recreate such an RNA complex by *in vitro* evolution.

#### 818 *From DNA to Last Universal Common Ancestor (LUCA)*

819 The origin of encoded protein synthesis appears the hardest step from RNA to  
820 modern life. By contrast, only smaller steps appear to be required to get to  
821 DNA and then to the LUCA. We (Poole et al. 2001) have discussed a series of  
822 steps: methylated-RNA; possibly a double-stranded RNA phase (but see later);  
823 ribose reduction to deoxyribonucleotides (giving DNA containing uracil,  
824 U-DNA); and the replacement of uracil (U) by thymine (T). The Darwin–  
825 Eigen cycle (Figure 1) is fundamental – more information allows more genes,

826 allowing increased accuracy of replication, allowing more genes, and so on.  
827 The advantage of replacing uracil by thymine is that higher accuracy replica-  
828 tion is expected. Cytosine in DNA deaminates spontaneously to uracil.  
829 Therefore it is hard for error-correcting enzymes to distinguish when a uracil in  
830 a sequence is 'real', or whether it is a mutation that needs to be corrected back  
831 to cytosine. On a different aspect, Forterre (2001) raises the possibility that the  
832 later steps of DNA evolution may have evolved more than once. He also  
833 suggests that early viruses could have an advantage from using new nucleotides  
834 (such as T) to help avoid host defense mechanisms. Later the new nucleotides  
835 could have been integrated into the host and allowed an increase in replication  
836 fidelity. Such mechanisms are incremental in that they allow smaller interme-  
837 diate steps, and it indicates additional selective forces that lead to increased  
838 complexity.

839 From of DNA to the LUCA there would again be many very small steps  
840 as new proteins arise (Figure 2). There are no conceptual difficulties here;  
841 they are the same processes that we observe today with gene duplication and  
842 divergence of the two copies. Similarly the maximum size of proteins in-  
843 creases through time, with the largest and most complex proteins being found  
844 in multicellular eukaryotes (Caetano-Anolles and Caetano-Anolles 2003).  
845 There is uncertainty over aspects of the later parts of the model. For  
846 example, Woese (2002) argues that even at the relatively late stage of the  
847 LUCA there was still extensive gene flow between organisms – 'primitive  
848 cellular evolution is basically communal'. This is a 'Garden of Eden'  
849 hypothesis (the lion lay down with the lamb) – all genes were cooperative,  
850 none were selfish.

851 This idealistic scenario is unlikely for at least two reasons. There are major  
852 advantages in an effective combination of genes staying together, perhaps on  
853 some form of pre-chromosome. Similarly, it is essential that any system be  
854 protected against invasion by parasitic sequences (see Boerlijst and Hogeweg  
855 1995). At a much earlier stage, perhaps in the RNA or RNP-world, it would  
856 have been harder to hold together good combinations of genes, and mixing of  
857 genes may have been harder to prevent. It is particularly as a counter to the  
858 Woese argument that I included under the Principles section the condition that  
859 selective pressures were as relevant in the past as today. Stopping the breakup  
860 of good combinations of genes, and restricting invasions by parasites, were as  
861 important then as now. It is certainly expected that effective mechanisms would  
862 evolve early to keep good combinations of genes together, and prevent inva-  
863 sion by parasitic genes.

864 For a quick summary of Themes 1 and 2, it suffices to say that we know that  
865 RNA carries out many catalytic reactions and control functions. There is a  
866 plausible and testable model to get from the RNA-world to protein synthesis,  
867 and that the Darwin-Eigen cycle is a strong driving force for a continued  
868 increase in replication fidelity and genome size. The major problem now is the  
869 origin of the RNA-world itself; the RNA-world was probably already complex  
870 in its metabolism.

**871 Theme 3 – are there alternatives to RNA?**

872 The RNA-world hypothesis is an excellent mid-point for the top-down and  
873 bottom-up approaches. If the chemists could get to an RNA-world, the biol-  
874 ogists could take over and extend along the continuum to modern life. Thus the  
875 RNA-world is an important reference point, and a target for the bottom up  
876 approach. However, RNA is unlikely to have been the first self-replicating  
877 molecule, and there are important issues with its origin in a prebiotic envi-  
878 ronment. In this section I simply ask whether there good reasons for  
879 just four bases in RNA (adenine, uracil, cytosine and guanine);  
880 these bases rather than other self pairing molecules;  
881 sugars (and ribose in particular) as part of the backbone of the molecule; and  
882 selection between optical isomers (D- and L- forms).

883 It is important to know how close we have to be to the target (RNA). Are  
884 there many alternatives that work well, or just a small number? If RNA is best;  
885 would some alternatives evolve easily toward it? Are there alternatives to our  
886 existing nucleotides, or to four nucleotides in RNA (is four really a sacred  
887 number in biology), and does the sugar have to be ribose?

**888 *Alternatives to A, C, G and U, and the numbers of nucleotides.***

889 Alternatives could either be completely different to nucleotides, or just alter-  
890 native nucleotides. Rebek (1994) gives an account of his work with comple-  
891 mentary self-replicating molecules, only one of which contains adenine. The  
892 other molecules are not nucleotides (one is a biphenyl molecule) but they are  
893 still self replicating in complementary pairs. An interesting aspect is that the  
894 system ‘evolved’ in that some molecules readily underwent self-replication and  
895 outcompeted others. Thus, in principle, molecules other than nucleotides are  
896 possible, though they are not synthesized so easily as adenine in the prebiotic  
897 world (see later). But an important principle is established; self-replication is a  
898 basic property of the appropriate molecules, a property that has been co-opted  
899 by living systems.

900 Why four? Given an even number of nucleotides for pairing, why not two,  
901 or six, or eight. After all, there are 20 amino acids, why not 20 nucleotides?  
902 The question may sound academic, but has flourished after Piccirilli et al.  
903 (1990) reported that an additional pair of nucleotides could be made syn-  
904 thetically – and could be incorporated into RNA- and DNA-like molecules.  
905 A nucleic acid with six nucleotides, not four – three pairs of nucleotides, not  
906 just two! The quantum mechanical calculations of Mac Dónaill and Broc-  
907 klebank (2003) suggest that two hydrogen-donor mismatches are required for  
908 accurate discrimination between pairs of nucleotides, so this reduces the  
909 number of base pairs that are feasible. It places an upper limit because it  
910 would be difficult to have an effective pairing system with high numbers of  
911 nucleotides.

912 At the lower end, Reader and Joyce (2002) use standard *in vitro* evolution  
 913 techniques (see earlier) and evolve a ribozyme with just two nucleotides. In this  
 914 case uracil was replaced by diaminopurine to pair with thymine, giving three  
 915 H-bonds between each pair of nucleotides. The resulting two-nucleotide RNA  
 916 ligase ribozyme functioned, but its catalytic ability was much weaker than the  
 917 original 4-base ribozyme from which it was derived. So there is a real question  
 918 whether four nucleotides is a genuine optimum. Could six nucleotides be better  
 919 than four, but nature did not find it before proteins ‘took over’ most catalysis.

920 Szathmáry (2003) reviews the problem, and from experiment, simulation and  
 921 theoretical calculations, it seems that four nucleotides outcompetes two, six or  
 922 eight. However, in simulations under some rates of mutation, six nucleotides  
 923 can evolve to a new structure faster than four (Gardner et al. 2003). But this  
 924 does not affect the conclusion that four nucleotides are more effective than two;  
 925 it only describes the phenomenon rather than explaining the greater efficiency  
 926 of four-nucleotide ribozymes.

927 Our suggestion (Gardner et al., in preparation) is that with only a single pair  
 928 of nucleotides, each sequence can fold in an extremely large number of ways  
 929 and still be close to the 3-D structure with minimum free energy (mfe). An  
 930 RNA-world is unusual in that the RNA molecule is both the genotype (the  
 931 actual sequence) and the phenotype (how the sequence folds in two and three  
 932 dimensions). Because RNA with just two nucleotides (one pair) can fold so  
 933 many ways, it appears that with a 2-letter code genotype does not specify a  
 934 consistent phenotype. In contrast, with four nucleotides there are fewer po-  
 935 tential mfe structures and therefore there is more consistency between a pri-  
 936 mary sequence of RNA, and its secondary structure. We think that this is the  
 937 best explanation at present for why RNA (and therefore DNA) has more than  
 938 just one pair of nucleotides, and that this idea merits further testing.

### 939 *Alternative sugars*

940 A well-recognized problem is that ribose is difficult to synthesize, and has the  
 941 chirality problem of mirror images around some carbon atoms. Are there  
 942 alternatives to ribose? (for example) Eschenmoser (1999) has synthesized  
 943 analogues of RNA with different sugars, crystallized them, and measured many  
 944 physical properties. Sutherland and Whitfield (1997) summarize some of this  
 945 earlier work and give the sugar analogue and the relative strength of base  
 946 pairing.

Ribose (RNA and DNA)	GC > AT
Glucose – homo-DNA	GC > AA ~ GG > AT
Allopyranosyl (6C)	AA ~ GG > GC, etc.

947 This means that for ribose and deoxyribose (RNA and DNA) the GC pair is  
948 the strongest, then the AT pair. However, for a nucleic acid based on glucose,  
949 although GC is strongest pairing, G also pairs quite strongly with itself.  
950 Similarly, adenine (A) pairs strongly with itself, even stronger than with  
951 T. With some sugars, the relative strength of base pairings varied with the  
952 sequence, not a desirable feature for a general system of pairing. Thus for  
953 complementarity of pairing, ribose is a particularly suitable sugar. Other sugars  
954 are possible. For example, Chaput and Szostak (2003) report that a TNA  
955 (T = trehalose, a four-carbon sugar) has some elementary properties of  
956 interest and could possibly be a precursor to RNA.

957 Some sugars give a nucleic acid with stronger binding between the comple-  
958 mentary strands than RNA, and these have sometimes been considered as  
959 'more suitable'. However, it is probable that high binding energy between the  
960 strands of dsRNA is an undesirable feature – rather, the specificity of com-  
961plementary pairing is more important. It is likely that in an RNA-world the  
962 positive and negative strands of RNA were kept separate – in the absence of  
963 proteins unwinding dsRNA would be extremely difficult. In the present (DNA)  
964 world there is a complex of proteins that cut and unwind dsDNA and then  
965 rejoin it, but this would not be possible in an RNA-world. In modern cells,  
966 dsRNA does occur, but tends to be used as a signal to destroy that sequence.  
967 Good complementarity of base pairing is expected to be the main requirement.

968 To summarize this section, it is not necessary to decide whether the com-  
969bination of ribose as the sugar and A, C, G and U (T in DNA) as the nucle-  
970otides is the optimal combination. We do not see any obviously better  
971 candidate than RNA, nor do there seem to be good alternatives for either  
972 sugars and/or nucleotides. If there were 'precursors' to RNA (which RNA  
973 eventually outcompeted) then this simplifies the situation in that a range of  
974 molecules could evolve toward RNA. In a sense the 'target size' for the bottom-  
975 up approach is larger. It is more productive at present to focus on the origin of  
976 RNA as a system that had all four of our properties for a living system, energy,  
977 biochemistry, cell organization and heritability.

#### 978 *Hot start/cold start?*

979 There are two additional properties, temperature and pressure, where the  
980 properties of RNA may limit the physical conditions for the origin of life. Did  
981 life originate on earth at high temperatures (such as around hydrothermal  
982 vents deep in the oceans – black smokers), or in relatively cold conditions?  
983 Knowing the answer to this question would focus further experiments. Previ-  
984ously, the most popular theory was a high temperature origin ('hot-start'), and  
985 was supported by the apparent placement of the 'Last universal common  
986 ancestor' (LUCA) of all living organisms among thermophilic bacteria (live  
987 above about 80 °C). However, an improved method of building evolutionary  
988 trees (Forterre and Philippe 1999) suggests that that apparent position of the

989 'root of the Tree of Life' was an artifact of the earlier phylogenetic methods  
990 used. Similarly, all modern hyperthermophiles use a reverse gyrase protein to  
991 stabilize DNA at very high temperatures. But this protein appears to be a  
992 fusion product from two existing enzymes used by all organisms; it is not  
993 one of the first proteins (Forterre 2001). In popular imagination we think of life  
994 originating in dramatic fashion in the bottom of the ocean where super-heated  
995 water ( $\gg 100\text{ }^{\circ}\text{C}$ ) gushes out. Unfortunately for popular imagination, at such  
996 high temperatures,

997 Ribonucleotides are unstable (the nucleotides break down, Levy and Miller  
998 1998),

999 RNA itself is unstable (the phosphate-sugar backbone breaks, Forterre 1995),  
1000 RNA does not fold into 2D and 3D structures (Moulton et al. 2000).

1001 For such reasons, some form of 'cold-start' seems a more likely starting  
1002 point (Bada and Lazcano 2002).

1003 This conclusion is reinforced because, in general, the higher the temperature  
1004 the harder it is to get ordered structures. Ice is highly ordered compared with  
1005 liquid water, which is highly ordered with respect to steam. In thermody-  
1006 namics, the decrease in order is measured as the change in entropy ( $\Delta S$ ) but is  
1007 negative with respect to temperature ( $-\Delta S$ ); higher temperatures increase this  
1008 negative value. With many millions of years available, the rate of a reaction is  
1009 not necessarily limiting, but it is critical to find conditions where order or  
1010 complexity increases. There is a fundamental issue here. Is the problem of the  
1011 origin of life basically a problem of increasing order (an entropy problem), or  
1012 primarily limited by rates of reactions – a chemical kinetics problem? Given  
1013 also the problems of RNA at high temperatures, my guess is that the origin of  
1014 life is more of an entropy problem (rather than kinetic), and that high  
1015 temperatures are unhelpful.

1016 How low a temperature? We really have little idea. At present we probably  
1017 would require temperatures below  $60\text{ }^{\circ}\text{C}$  for the stability of RNA and its  
1018 precursors. But if the origin of life is an entropy problem then we cannot  
1019 exclude temperatures around  $0\text{ }^{\circ}\text{C}$ . There is a definite group advocating sub-  
1020 zero temperatures that occur in concentrated aqueous solutions within sea ice  
1021 and recent results in sea ice are encouraging (Trinks et al. 2003). The freezing of  
1022 sea water, giving a concentrated solution in the unfrozen water, can (Orgel  
1023 2004b) lead to high levels of adenine from HCN (see later). These very low  
1024 temperatures need exploring.

1025 *Open question: atmospheric pressure or high pressure?*

1026 However, the black smoker/hydrothermal vent suggestion raises another  
1027 question. Would it be easier for early life to develop at around one atmosphere  
1028 pressure – or under pressures of hundreds or thousands of atmospheres at the  
1029 bottom of the ocean? There are also 'cold smokers' where chemically-rich

1030 water comes up from the ocean floor. Alternatively, given a cold start scenario,  
1031 there will be places at sea-level at one atmosphere pressure in sea-ice (see above  
1032 paragraph) where there will be many highly ordered 'cells' (see later) that may  
1033 be advantageous.

1034 The question comes down to fundamental issues such as the stability of  
1035 nucleotides at high pressures (including deamination of cytosine). Similarly, do  
1036 two monomers (ribonucleotides) occupy a larger volume than a dimer? If so,  
1037 higher pressures might then be favorable to polymerization. Is nucleotide  
1038 pairing more stable at high atmospheric pressures? There is a need here for  
1039 fundamental research by physical chemists. A similar question, whether sea  
1040 water is the optimal medium for early stages of the origin of life was considered  
1041 by Monnard et al. (2002). Their results were that sea water inhibits both  
1042 membrane self-assembly and RNA synthesis. Clearly further experiments are  
1043 required to search for optimal conditions.

1044 To conclude this third section, it appears that there are no easy alternatives  
1045 to RNA. In an important sense this is good news. If there are other non-RNA  
1046 precursors (close to RNA, say with a different sugar such as trehalose), they are  
1047 likely to have been out-competed by RNA. Thus, the RNA-world might be  
1048 attainable through a variety of different routes; there need not be only a single  
1049 route to an RNA-world. Such a conclusion does not imply that other forms of  
1050 life were not possible, just that of the ones that might be possible in principle,  
1051 the RNA-based form was easier to find.

#### 1052 **Theme 4 – how to get to the RNA-world**

1053 At present, getting from gases and other small molecules to an RNA-world  
1054 appears the hardest task for the origin of life – but probably wasn't. On current  
1055 wisdom, it may have taken a mere 100 million years for RNA and DNA-based  
1056 living systems to appear on earth, but another 3 billion years to get multicel-  
1057 lular land plants and animals. Maybe getting to an RNA-world is not as hard  
1058 as evolving multicellular land animals? Taking the time-to-solve-a-problem as  
1059 the measure of complexity is simplistic, but is used to indicate that researchers  
1060 should persist with trying to understand these early chemical phases. (The  
1061 reasoning makes humans look easy, only about 5 million years from a  
1062 common ancestor with chimps.)

1063 Why does it appear so hard to get to RNA? How complex was the RNA-  
1064 world? Did it have all basic metabolism and organization (including mem-  
1065 branes)? From the discussion thus far we conclude that an intermediate stage  
1066 RNA-world explains a large number of observations, and that there is a large  
1067 body of experimental evidence of the catalytic abilities of RNA (ribozymes).  
1068 Furthermore, it is possible to make plausible models that take us from an RNA  
1069 world to the evolution of protein synthesis, to DNA synthesis, and to a last  
1070 universal common ancestor (LUCA). Even better, the models lead to testable  
1071 predictions. In the third section we find the number of alternatives to RNA to

1072 be limited. But the target does not have to be just RNA, as a range of similar  
1073 molecules could lead to RNA. Are we missing something obvious?

1074 *Origin of the small organic molecules used in life*

1075 In reviewing what we know of the origin of small organic molecules of living  
1076 systems, we have two complementary approaches;

1077 chemical simulations of possible prebiotic conditions (the test-tube approach),  
1078 and

1079 theoretical predictions from basic chemical properties. (What molecules are  
1080 expected to occur early, and where are they likely to be synthesized?)

1081 The results are compared to the basic biochemicals found in living systems.

1082 In very early studies, Oparin (see 1957) tried many mixtures of chemicals in  
1083 order to find what biological properties would develop in fairly simple systems,  
1084 in particular gel-like microbodies (coacervates). Although some interesting  
1085 physical properties of such systems were found he appeared, from our current  
1086 viewpoint, to make little progress - partly because the basic genetic and bio-  
1087 chemical problems were not understood at the time. However, the properties of  
1088 coacervates may turn out to be important as part of the 'organization' of cells,  
1089 we just don't have a theoretical framework to interpret them.

1090 By far the best-known work is on chemical simulations. As is well known, in  
1091 1953 Stanley Miller (see Bada and Lazcano 2003) reported the first chemical  
1092 simulations that are useful from our current understanding. He passed electric  
1093 discharges through a possible primeval atmosphere (containing methane [CH<sub>4</sub>]  
1094 and ammonia [NH<sub>3</sub>]) and found amino acids among the products! Many  
1095 researchers, including Oró, Ferris, Ponnampereuma, and Orgel, followed this  
1096 up; a recent review is Orgel (2004a). A startling finding was that the nucleotide  
1097 adenine can be formed by the polymerization of hydrogen cyanide (HCN, one  
1098 of the active chemical intermediates in the prebiotic experiments, see Suther-  
1099 land and Whitfield 1997 Figure 4 ■Au: Please check the placement of figure  
1100 4■). This is particularly striking. Adenine, as a component of ATP is a central  
1101 molecule in energy transfer reactions, is a component of coenzymes (such as

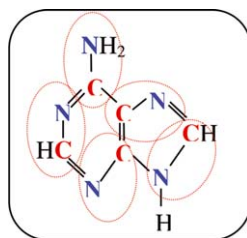


Figure 4. Adenine, as formed from five HCN molecules.

1102 FAD, NAD), and is part of both RNA and DNA. Thus basic building blocks  
1103 of life occur in simple chemical simulations of prebiotic chemistry. There is no  
1104 guarantee that this is the actual process by which adenine was made in  
1105 prebiotic systems, but at least adenine is likely to have been present and there is  
1106 a well-established chemical pathway for its synthesis (Orgel 2004b).

1107 We all accept the importance of the chemical simulation approach, but as a  
1108 theoretical biologist I also want an analysis of what small molecules are ex-  
1109 pected to occur early on the earth. Were small organic acids, amino acids,  
1110 nucleotides, co-factors, and/or sugars among the first molecules in early pre-  
1111 biotic reactions? Were these molecules more likely to be formed in aqueous or  
1112 gaseous phases, or at interfaces (including clays)? At higher or lower temper-  
1113 atures? At around one atmosphere pressure, or at high pressures (such as at the  
1114 bottom of oceans)?

1115 Returning to the early Miller experiment, we could consider the precise  
1116 conditions of the experiments and the precise composition of the starting  
1117 chemicals, and then argue about the details. Alternatively, we could look at the  
1118 fundamental principles; the experiment demonstrates that known chemical  
1119 reactions lead to small molecules that are of biological interest. I would argue  
1120 (this week at least) that the details of the original Miller experiment on forming  
1121 amino acids may not be of major interest for the early origin of life – amino  
1122 acids may not have been that important so early on. On the other hand, when  
1123 considering the principles, the Miller experiment is intellectually outstanding in  
1124 illustrating how known chemical reactions lead to small molecules of biological  
1125 relevance. But which molecules do we expect to be synthesized first?

#### 1126 *Gas phase/aqueous phase*

1127 One model is that the small biochemical molecules formed either in reactions in  
1128 the atmosphere (and then were absorbed into the ocean), or in an extrater-  
1129 restrial environment and were carried to the earth during its accretionary phase  
1130 (see comments in Orgel 1998). For convenience, I will include both ideas as a  
1131 'gas phase' origin, even though they assume that later stages continued in the  
1132 ocean. These models focus particularly on small molecules that contain  
1133 nitrogen, especially amino acids, though that may not be essential to a gas  
1134 phase origin.

1135 An alternative to the gas phase is a completely aqueous origin for the first  
1136 biochemicals; in general this gives less emphasis to nitrogen-containing mole-  
1137 cules at the earliest stages of a prebiotic system. The ideas of Wächtershauser  
1138 (1992) are the best known, but I will base my analysis of the first biochemicals  
1139 on that of Morowitz (1992) and Morowitz et al. (2000). I will take for granted  
1140 the main chemical elements involved – carbon, nitrogen, oxygen, and phos-  
1141 phorus, see the discussion in Morowitz (1992, Ch. 10). The next step is to  
1142 decide how to predict which molecules will occur in prebiotic systems.  
1143 Morowitz et al. (2000) suggest that the most likely molecules will be:

1144 small (no more than six carbon atoms),  
 1145 water soluble, and  
 1146 have low heats of combustion (therefore are relatively easy to form chemi-  
 1147 cally).

1148 The authors start with a list of over 3.5 million organic (carbon-based)  
 1149 compounds and select those with no more than six carbons, leaving 2790 (a  
 1150 thousand-fold reduction). Water solubility was measured by the relative  
 1151 amounts of a compound in a water/octanol mixture (a standard technique for  
 1152 comparing water versus lipid solubility). Other minor rules were applied  
 1153 including stability, the relative ease of formation, and being chemically reac-  
 1154 tive. The final list is 153 compounds – the list includes all the compounds  
 1155 involved in the tricarboxylic acid cycle. This cycle is shown (as the reductive  
 1156 TCA cycle, and as a potential flow reactor) in Figure 5, with the name of the  
 1157 organic acid following its number of carbon atoms).

1158 Just like the original Miller experiment, the result is striking. The small  
 1159 molecules involved in metabolism in modern-day living cells are precisely the  
 1160 molecules that are likely to form in an early earth. This is considered a ‘uni-  
 1161 versal’ of biology (Smith and Morowitz 2004). The conclusion only considers  
 1162 the earliest stages and molecules lacking nitrogen. But as the authors point out,  
 1163 such nitrogen-containing molecules can form from the molecules of the  
 1164 reductive TCA cycle (rTCA), or from related molecules no more than 1 or 2  
 1165 reaction steps away. A standard chart of biochemical reactions shows many  
 1166 such possibilities. But to go earlier still, where do the molecules of the rTCA  
 1167 cycle come from. The best guess at present would be the simple ‘free lunch’  
 1168 reaction (Martin and Russell 2003)

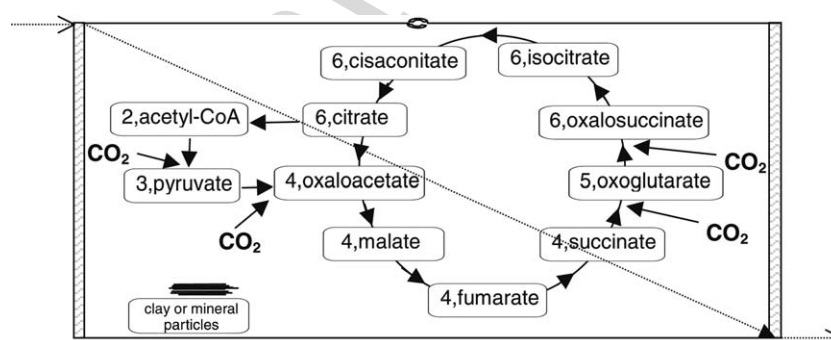


Figure 5. The Reductive Tricarboxylic Acid Cycle (rTCA) in a potential flow-reactor. There is a net synthesis of molecules, given an input of  $\text{CO}_2$  and energy. Some form of compartmentation is indicated by membranes on the left and right, together with an energy gradient from left to right. The model combines an energy source, basic biochemical molecules, and compartmentation. Potential metallic or clay catalysts can be included in the reactor.

1170 In a reducing atmosphere the equilibrium strongly favors acetic acid and  
1171 water. Wächtershauser (1992) has the same basic cycle but adjusted for the  
1172 high sulfur and iron content expected on the early earth (Anbar and Knoll  
1173 2002).

1174 Basically, this approach leads eventually to a 'metabolism first' theory, and  
1175 we will come back to it – it is the basis of much current thinking. However, it is  
1176 first necessary to consider some additional features such as polymerization and  
1177 organization.

#### 1178 *Polymerization, organization and energy*

1179 In the above section, the treatment only covers possible origins of basic bio-  
1180 chemistry, and we still need to include three other aspects of the prebiotic  
1181 world - polymerization, compartmentation and energy sources. With respect to  
1182 polymerization of amino acids or nucleotides, important principles are estab-  
1183 lished by demonstrating the formation of short (unordered) peptides by a series  
1184 of 'drying cycles'. These involved a repeated series adding a solution of amino  
1185 acids to clays (particularly montmorillonite, see Ertem 2004), evaporating the  
1186 solution, and heating. This cycle of adding a solution of amino acids, drying,  
1187 and heating, is repeated several times. In each cycle one additional amino acid  
1188 may be added to the growing peptide.

1189 When heated dry (with amino acids absorbed onto a clay surface) any water  
1190 formed during the formation of a dimer (with its peptide bond) is lost to the  
1191 atmosphere, driving the reaction toward synthesis. Prior adsorption of amino  
1192 acids onto the clay surface reduces entropy costs of the polymerization reaction  
1193 because the amino acids are already partly ordered (on the clay surfaces) rela-  
1194 tive to their free movement in solution. In contrast to reactions on clay sur-  
1195 faces, in solution the high water concentration drives the polymerization  
1196 reaction towards hydrolysis of peptides, and the amino acids are fully free to  
1197 diffuse and rotate in solution. This establishes again that basic chemical  
1198 principles are being followed in such prebiotic simulations. However, to  
1199 achieve polymerization in an aqueous solution, whether of nucleotides or  
1200 amino acids, is a major difficulty. Nevertheless, as outlined below, we still want  
1201 a 'single compartment' answer.

1202 'Life as we know it is cellular', is a useful slogan that introduces compart-  
1203 mentation and membranes. Appropriate molecules for membranes are ex-  
1204 pected to be harder to find in prebiotic systems, but suitable longer chain  
1205 molecules are found even in organic material extracted from meteorites (see  
1206 Deamer et al. 2002). Given the absence of proteins in the prebiotic environ-  
1207 ment, lipid bilayers are the best-known model. Such bilayer membranes form  
1208 spontaneously in the right environment because of the lower free energy of  
1209 having the hydrophobic ends of two lipids together. The membranes can be a  
1210 layer between compartments in experimental systems, spherical membranes

1211 separating two phases (such as surrounding gels), or spherical liposomes (with  
1212 aqueous phases inside and out).

1213 Compartmentation and membranes are certainly expected to be important.  
1214 Many energy sources are used by living systems (see Martin and Russell 2003),  
1215 all of which use proton gradients across membranes. Apart from a few sub-  
1216 strate-level phosphorylation reactions, the energy currency in biology is proton  
1217 gradients – they are as universal as proteins and nucleic acids. Morowitz (1981)  
1218 discusses the relevance of proton gradients to the origin of life (though in a  
1219 photosynthetic perspective) and concludes that a proton gradient is the only  
1220 possibility. Electrons, for example, need a quite different (metallic) structure  
1221 that is not found in living systems, nor is transport of large ions likely to be a  
1222 solution.

1223 Turning from the experimental to the theoretical, in addition to the observed  
1224 requirement of living systems for membranes, Zintaras et al. (2002) report  
1225 theoretical results where more-complex molecules can evolve in compartments.  
1226 The effect comes from both increasing cooperativity (good replicators are more  
1227 likely to be copying other good replicators), and also by reducing the chance of  
1228 parasitism. The latter may be unrelated molecules that, for example, could be  
1229 easily copied but do not reciprocate by replicating other molecules. Comple-  
1230 mentary to these theoretical results are experimental systems of authors such as  
1231 Hanczyc et al. (2003) where, for example, the clay particles are included with  
1232 liposomes. This is an important step to a flow reactor, But perhaps it is not  
1233 sufficiently controllable to be a useful model? Thus for both theoretical and  
1234 empirical reasons, and from considerations of energy use (proton gradients), it  
1235 appears that membranes need to be considered very early in prebiotic stages.  
1236 One suggestion for early ‘protocells’ has them occurring inside the small spaces  
1237 of volcanic rock (Martin and Russell 2003) or other subterranean location  
1238 (Trevors 2003). In general then, our conclusion is that energy sources,  
1239 compartmentation and membranes, are critical for early prebiotic systems.

#### 1240 *A new beginning?*

1241 Over the past 50 years most of the work in the prebiotic realm appears to  
1242 follow the path of synthetic chemists. This is a linear model of chemical syn-  
1243 theses, starting with a smaller molecule, and adding moieties sequentially until  
1244 the final product is reached. Why has this approach not been more successful  
1245 over the last 50 years? Much has been learned about individual reactions,  
1246 classes of reactions, and polymerization of amino acids on the surfaces of clays  
1247 during drying cycles. But it has not lead to any major discovery such as a likely  
1248 early metabolism, or how to get to an RNA-world. What are we missing?

1249 Perhaps the linear model is not the right approach. The linear approach  
1250 (including the Darwin–Eigen cycle) is a powerful model for later stages of the  
1251 origin of life, after life has a genetic inheritance system. It leads from a simple  
1252 RNA-world, to a complex RNA-world with an RNA-triplicase as a high

1253 fidelity RNA polymerase reaction. This is followed by the advent of protein  
1254 synthesis, perhaps methylated RNA (meRNA), to DNA, and onwards. At  
1255 these later stages the linear (incremental) model is both plausible and consistent  
1256 with known principles. In contrast, for the prebiotic stages of the origin of life,  
1257 the linear model (with a large number of sequential chemical syntheses in the  
1258 right order eventually leading to RNA) does not seem to give equivalent new  
1259 and exciting discoveries.

1260 Over the past few years an alternative to the linear scenario for the prebiotic  
1261 arena is being considered; one where the three requirements of energy,  
1262 metabolism, and compartmentation (including membranes) are considered  
1263 together. We need to be able to consider thousands to millions of possible  
1264 reactions simultaneously, just like the *in vitro* evolution experiments where  
1265 routinely  $> 10^{13}$  variants of RNA sequences can be tested simultaneously; we  
1266 need an equivalent for searching for metabolic cycles. More like combinatorial  
1267 chemistry perhaps. The general idea has a long history. For example, Ninio  
1268 (1982, pp. 87–89) suggested making peptides at random, separating them by 2-  
1269 D chromatography, and searching for catalytic activity; he attributes the idea  
1270 to L. Orgel. When the focus changed from proteins to RNA it was used  
1271 successfully for evolving ribozymes.

1272 A flow reactor is a good start to simulate the problem for metabolism, see  
1273 Figure 5 for a rough idea. In this example, there is a central metabolic com-  
1274 partment that includes metal ions, it is bounded by membranes, and there is a  
1275 strong energy gradient from left to right. Such an idealized cartoon is intended  
1276 to be general, not to show particular chemicals or energy sources. However, a  
1277 critical feature is a metabolic cycle, in this case the reductive tri-carboxylic acid  
1278 (rTCA) cycle. Cycles are particularly interesting from the viewpoint of non-  
1279 equilibrium thermodynamics (see Glansdorff and Prigogine 1971), because they  
1280 lead to a high rate of energy dissipation down a steep energy gradient. A system  
1281 that ‘dissipates’ energy faster will have cycles turning faster, incorporating  
1282 additional chemicals (such as  $\text{CO}_2$ ) into the system. Even at this prebiotic stage  
1283 it is likely that there is selection between purely chemical systems, in the  
1284 manner included in the systems studies by Rebek (1994). In this sense the  
1285 system evolves, though as pointed out by different authors (e.g. Sowerby and  
1286 Petersen 2002; Chen et al. 2004) it is not yet a genetic system. I call this  
1287 pre-genetic evolution.

1288 It is not intended that a flow reactor ‘mimic’ nature precisely; nor simulate a  
1289 particular set of plausible conditions on the early earth. Rather, the intent is to  
1290 examine basic principles in a controllable way, and find if there are any likely  
1291 conditions where a set of self-sustaining reactions occur – given a membrane  
1292 bounded structure, a steep energy gradient, and a supply of precursors.  
1293 Hordijk and Steel (2004); also Mossel and Steel (2005) show that autocatalytic  
1294 cycles are likely to form in a diverse chemical system. They assumed initially  
1295 that each chemical could be both a reactant and a catalyst, but metal ions may  
1296 be the catalysts though not otherwise participating in the cycles. Thus auto-  
1297 catalytic cycles are reasonable mathematically, and thermodynamically they

1298 are advantageous. Some of the sets of conditions to try could be those  
1299 described in Eschenmoser (1999) and Martin and Russell (2003).

1300 We need to know if there are structured systems where, say, a reverse TCA  
1301 cycle can arise naturally, and whether the next layers of small molecules will  
1302 then appear, especially those containing nitrogen. Concepts from biomimetic  
1303 chemistry (Sutherland and Whitfield 1997) are important here, the idea that  
1304 pathways found in biochemistry are the most straightforward ways to more  
1305 complex molecules; pathways that were found early in protometabolism.  
1306 Maybe the proposed flow reactor is too simple, trying to put organization and  
1307 energy gradients (plus metabolism) back together in one step. However, we  
1308 need to find whether the reactions from the very earliest stages of the origin of  
1309 life were autocatalytic. The conclusion here is that we need to try an organized  
1310 system, with compartmentation by membranes, a steep energy gradient, and a  
1311 setup that may allow cycles. This might combine our knowledge from 50 years  
1312 of chemical simulations in a manner where new processes could arise.

### 1313 **Summary and conclusions**

1314 There is still a significant gap between what has been achieved by the top-down  
1315 and bottom-up approaches, though nothing like as large as it was 30 years ago.  
1316 Most researchers now consider the problem as solvable – say in another 10–  
1317 20 years. This is far more optimistic than the view of 40–50 years ago when  
1318 most scientists would have considered the problem inherently unsolvable,  
1319 forever beyond the ability of the most powerful science then imaginable.

1320 It is important to examine the logic behind the different conclusions. Life is a  
1321 natural property of matter; its origin is a scientific problem that was, in refuting  
1322 continuous spontaneous generation, discovered by science. Instead of a sta-  
1323 tistical ‘all at once’ answer, we are looking for a mechanistic, step-by-step  
1324 approach that follows Darwin’s principle of continuity (which is a version of  
1325 the standard scientific principle of microscopic reversibility). In addressing the  
1326 problems we need to consider both the basic theoretical work on what is  
1327 possible, as well as the experimental simulations.

1328 I have considered four main themes in order to look for a continuous set of  
1329 intermediates. The RNA-world hypothesis (though qualitative) is a powerful  
1330 scientific theory that is consistent with numerous phenomena, many discovered  
1331 after the development of the theory. It removes the apparent chicken-and-egg  
1332 problem between DNA and protein. The hardest step from an RNA-world to  
1333 modern life appears to be the origin of protein synthesis, and here the tripli-  
1334 case/proto-ribosome theory, adding triples of nucleotides to a replicating  
1335 RNA, is so far the only plausible theory. It is plausible in the sense it is both  
1336 consistent with our knowledge (including selective forces) and that at least  
1337 some aspects are testable. It separates the origin of the code into the questions  
1338 of its length (three) and then of the specific triplet code (which is suggested to  
1339 be at least partially established well before protein synthesis evolved). The

1340 length of the code is suggested to arise from a ratchet mechanism that  
1341 overcomes the problem of processivity.

1342 Later steps to get from an RNA-world, to an RNP-world, to modern  
1343 biology appear more straightforward. The Darwin-Eigen cycle is likely to be a  
1344 powerful selective force in the many steps from an RNA-world to the modern  
1345 biological world. However, I would like to know more of the specific situations  
1346 when it should lead to an increase in complexity (total information coded). We  
1347 do not know of good alternatives to an RNA-world. It is likely that there may  
1348 have been precursor stages to RNA with simpler sugars, or just two nucleo-  
1349 tides. However, we do not yet know of alternatives that were likely to arise  
1350 naturally and which would have been better.

1351 Thus the focus is still on how to get to RNA. For these prebiotic stages  
1352 (before RNA) we may need to solve metabolism, energy gradients, and com-  
1353 partmentation simultaneously – thus the intense interest in some form of flow  
1354 reactor. If an autocatalytic cycle could arise, we would be major steps ahead in  
1355 our understanding. The most likely physical conditions for the origin of life  
1356 require further clarification, especially in regard to temperature, pressure,  
1357 chemical composition and energy sources. If its origin is an entropy (infor-  
1358 mation) problem, rather than a kinetic or energy problem, then low-temper-  
1359 ature conditions warrant much more attention. My favored analysis at present  
1360 is ‘metabolism, energy and organization first, metabolism makes RNA, RNA  
1361 makes protein, and protein makes DNA’.

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