Ribozymes: **Building the RNA world**

Gerald F. Joyce

The isolation of an RNA enzyme with RNA replicase activity, at present only a hypothetical molecule, is considerably closer following the recent demonstration of RNA-catalyzed polymerization of nucleoside triphosphates.

Address: Departments of Chemistry and Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, USA.

Current Biology 1996, Vol 6 No 8:965–967

 ISSN 0960-9822

For nearly thirty years, nucleic acid biochemists have dreamed of a very special RNA molecule, still hypothetical, which is thought to have played a key role in the early history of life on earth and may enable the production of life in the laboratory. That molecule is an RNA enzyme, or ribozyme, with the ability to catalyze the replication of RNA molecules, including copies of itself. All known terrestrial life is based on DNA as the primary genetic material and protein as the chief agent of catalytic function. It is widely believed, however, that DNA–protein-based life was preceded by RNA-based life, with RNA serving as both the genetic material and principal catalyst in an era usually referred to as the 'RNA world' [1]. There are many uncertainties concerning how the RNA world arose, what degree of metabolic complexity it attained, and how it eventually gave way to DNA and proteins [2,3]. A defining feature of the RNA world is that it contained RNA molecules that were capable of undergoing Darwinian evolution based on natural selection. This requires that the RNA molecules were replicated efficiently and accurately, in a reaction that was catalyzed by the RNA itself.

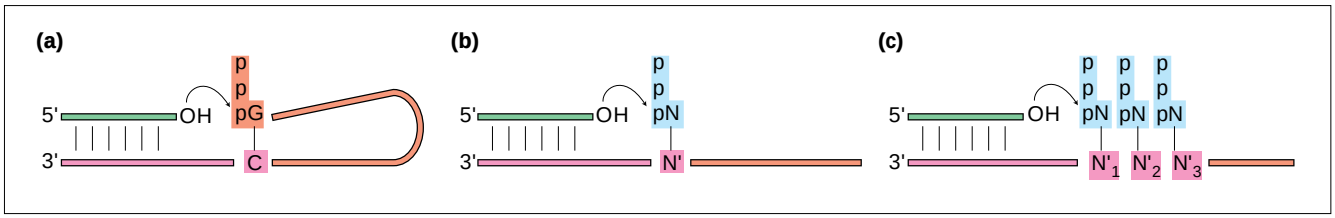
RNA is known to be capable of various catalytic functions, including phosphoester transfer chemistry similar to what would be required of an RNA replicase. Relying on *in vitro* evolution techniques, David Bartel and Jack Szostak [4] pushed this analogy further by developing an RNA enzyme that catalyzes the template-directed condensation of an oligonucleotide 3′-hydroxyl and an oligonucleotide 5′-triphosphate. This reaction results in the formation of a 3′,5′-phosphodiester linkage between the two oligonucleotides, with concomitant release of inorganic pyrophosphate, similar to the reaction catalyzed by an RNA-dependent RNA polymerase. More recently, Eric Ekland and David Bartel [5] have taken another major step by using a related RNA enzyme to catalyze the template-directed polymerization of mononucleoside 5′-triphosphates (NTPs), in a reaction that proceeds with remarkable efficiency and surprisingly high fidelity. An RNA replicase that allows RNA-based Darwinian evolution to occur is not yet at hand, but it may soon be within reach.

Before describing the results of Ekland and Bartel in more detail, I will summarize the general features that are required of an RNA enzyme that acts as an RNA replicase enabling Darwinian evolution. First, it is essential that RNA replication be template-directed. The specific ordering of subunits within a preformed RNA polymer must be reflected in the synthesis of a complementary RNA product. The nature of this complementarity need not be Watson–Crick pairing, although that would seem to be the most obvious possibility. Second, the reaction must be energetically favorable. It is possible to synthesize RNA polymers through an energy-neutral dismutation reaction, in which every monomer that is added to the growing chain comes at the expense of a monomer that is removed from some other chain. This is a losing proposition, however, if long polymers are required for the embodiment of a sophisticated RNA enzyme. Nucleotide polymerization reactions in biology usually depend on the cleavage of a phosphoanhydride (such as the α–β linkage of NTP) to drive the formation of a phosphodiester. Chemists typically turn to more highly activated monomers, such as nucleoside 5′-phosphorimidazolides, to achieve high reactivity in favor of polymerization. But this reactivity comes at a price because monomer addition is then less dependent on the template and the system is more prone to side reactions.

The ideal nucleotide polymerization reaction is one that is energetically favored but kinetically difficult. In that case, no appreciable reaction occurs except in the context of the template and a suitable catalyst. This is a third useful, though not essential, feature of RNA replication. The template-directed polymerization of NTPs again fits the bill because this reaction does not occur to an appreciable extent in the absence of a catalyst. If polymerization is kinetically difficult, then an efficient catalyst is required for monomer addition to proceed at an acceptable rate relative to spontaneous decomposition. The ribozyme of Ekland and Bartel catalyzes the template-directed condensation of an oligonucleotide 3′-hydroxyl and an NTP at a rate of 0.3 min^{-1} [5]. The uncatalyzed rate of reaction is too slow to measure, but the catalyzed rate is more than 10^6-fold increased compared with the uncatalyzed rate of ligation of two oligonucleotides.

A fourth essential feature of an RNA enzyme with RNA replicase activity is that it operate with sufficiently high fidelity to produce accurate copies of RNA molecules that

Figure 1



Conversion of an RNA ligase to an RNA polymerase. (a) Template-directed condensation of two oligonucleotide substrates (green and orange), catalyzed by an RNA enzyme. (b) Condensation of an oligonucleotide (green) and an NTP (blue). (c) Successive addition of three NTPs (blue). The template is colored pink.

are at least as long as itself. There is an extensive theoretical and experimental literature on this subject (see, for example, [6]). As a good rule of thumb, maintenance of genetic information over successive rounds of replication requires that the error rate not exceed the inverse of the number of subunits that comprise the polymer being replicated. Thus, an RNA enzyme that contains 100 nucleotides must be replicated with an error rate of not more than about 1 % per nucleotide (fidelity ≥ 99 %). The ribozyme employed by Ekland and Bartel, which contains 98 nucleotides, has an overall error rate of 8 % under optimal conditions [5]. This is far better than would be expected based on the fidelity of Watson–Crick pairing alone, but allows propagation of an RNA containing only about 12–13 nucleotides.

Finally, the RNA replicase must be able to produce a complete copy of a template that is at least as long and complex as itself. It must be able to overcome typical regions of template secondary and tertiary structure, and must copy both the template strand and its complement. Generality with respect to the length and sequence of the template is likely to be the most difficult property for an RNA replicase to attain, especially if high catalytic rate and high fidelity are to be attained as well. This is where the RNA enzyme of Ekland and Bartel begins to falter. It can copy as many as three residues of the template with high fidelity, irrespective of the sequence, but it cannot do more.

The RNA enzyme used for the template-directed polymerization of NTPs was originally developed as an RNA ligase for the condensation of two oligonucleotides [4,7]. Six residues at the 3′ end of one oligonucleotide substrate and one residue at the 5′ end of the other are held in close proximity by binding to a complementary template (Fig. 1a). The residue at the 5′ end of the second substrate bears the 5′-triphosphate moiety that drives the condensation reaction. Ekland and Bartel replaced the second oligonucleotide by an NTP and varied the opposing template residue, using either C, U, G or A to direct the incorporation of either GTP, ATP, CTP or UTP, respectively (Fig. 1b). In each case, the reaction proceeded efficiently and with high fidelity [5]. Emboldened by this success, they then inserted two additional template residues adjacent to the one that bound the NTP. Now the RNA enzyme could direct the incorporation of three successive NTPs, still with high fidelity, but with significantly reduced efficiency (Fig. 1c). Unfortunately, the RNA enzyme could not be made to accommodate more than three template residues for binding NTPs. This is not surprising, however, considering that the enzyme had been evolved to carry out a single condensation reaction with two oligonucleotide substrates.

It is clear what needs to be done. The RNA enzyme must either be redesigned or ‘taught’, through *in vitro* evolution, to accept a template of indeterminate length. The template must be an external RNA molecule of variable sequence that can be copied by the RNA-catalyzed polymerization of complementary NTPs. The fidelity of the reaction must be improved so that the error rate is no more than about 1 %, and the efficiency must be maintained over repeated nucleotide additions. Although this is a tall order, the results of Ekland and Bartel suggest it is not unreasonable.

Once an RNA enzyme with RNA replicase activity is in hand, the dreaming stops and the fun begins. An RNA enzyme with the ability to catalyze RNA replication would allow Darwinian evolution to operate in a self-sustained manner. The enzyme would produce additional copies of itself, which in turn would do the same, and so on until the supply of NTPs was exhausted. If the experimenter provided an ongoing supply of NTPs, for example by serial transfer or in the context of a flow reactor, then replication would continue indefinitely. As a consequence of the imperfect fidelity of the replicase, mutations would arise, some of which might prove to be beneficial with respect to replicase function. The evolving population of RNA enzymes would be expected to develop specificity for substrates that resemble themselves, and to replicate those substrates with increasing efficiency and fidelity.

Evolutionary innovation would continue so long as selectively advantageous mutations were obtainable. Would this process ever lead to something as impressive as current biology? Probably not. But it would serve as a working model of the RNA world and provide a powerful tool for studying molecular evolution and RNA catalysis.

The demonstration of an RNA enzyme with replicase activity would no doubt be taken as further evidence for the existence of an RNA world during the early history of life on earth. It should be noted, however, that a process of Darwinian evolution would be required to develop such a molecule in the laboratory. Thus, the question would remain as to how evolution could have begun in the first place. For the answer to that question, one must look to prebiotic chemistry.

References

1. Gilbert W: The RNA world. *Nature* 1986, 319:618.
2. Joyce GF: The rise and fall of the RNA world. *New Biologist* 1991, 3:399–407.
3. Joyce GF, Orgel LE: Prospects for understanding the origin of the RNA world. In *The RNA World.* Edited by Gesteland RF, Atkins JF. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 1993:1–25.
4. Bartel D, Szostak J: Isolation of new ribozymes from a large pool of random sequences. *Science* 1993, 261:1411–1418.
5. Ekland EH, Bartel DP: RNA-catalyzed RNA polymerization using nucleoside triphosphates. *Nature* 1996, 382:373–376.
6. Eigen M: Self organization of matter and the evolution of biological macromolecules. *Naturwissenschaften* 1971, 58:465–523.
7. Ekland EH, Szostak JW, Bartel DP: Structurally complex and highly active RNA ligases derived from random RNA sequences. *Science* 1995, 269:364–370.