

Evolution Based on Design-by-Contract: Origin of Life through an abiotic double-stranded RNA world

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Abstract

It is generally believed that life first evolved from ssRNA (ssRNA) that both stored genetic information and catalyzed the reactions required for self-replication. By modeling early genome evolution on the engineering paradigm design-by-contract, an alternative scenario is presented in which life started with the appearance of double-stranded RNA (dsRNA) as an informational storage molecule while catalytic single-stranded RNA was derived from this dsRNA template later in evolution. Double-stranded RNA can be formed abiotically by hybridization of oligoribonucleotides that are subsequently non-enzymatically ligated into a double-stranded chain. Thermal cycling driven by the diurnal temperature cycles can replicate this dsRNA when strands of dsRNA separate and later rehybridize with oligonucleotides that are subsequently ligated to reform dsRNA. Temperature-dependent partial replication of specific regions of dsRNA can produce the first template-based generation of catalytic ssRNA, similar to the developmental gene transcription process. Thus, the dsRNA-first scenario can be implemented by gradual processes based on abiotic ligation of oligonucleotides and hybridization of complementary nucleotides. Further transition from a dsRNA to a dsDNA world can be based on minor mutations in template and substrate recognition sites of an existing RNA polymerase. Therefore, by defining evolution as an expanding system of functionalities in which existing interfaces remain intact, the 'dsRNA first' hypothesis provides a relatively simple gradual evolutionary scenario for the origin of Life.

Introduction

The evolution of life and the origins of DNA and RNA as the carriers of information are still a mystery. It has been proposed that the DNA world was preceded by an RNA world in which RNA fulfilled a role both as the information carrier and as the catalyst of early chemical processes (Joyce, 1989; Unrau and Bartel, 1998). The general idea is that from a pool of random strings of RNA, ribozymes would emerge with a primitive RNA polymerase activity and, in this way, RNA could provide its own replication machinery (Inoue and Orgel, 1983; Dworkin et al., 2003; Been and Cech, 1988; Wilson and Szostak, 1999; McGinness and Joyce, 2003; Johnston et al., 2001). The transition from a ssRNA world to the dsDNA world is thought to have arisen by a reverse transcriptase activity that copied an RNA template to dsDNA, possibly by a dsRNA intermediate (Leipe et al., 1999; Poole et al., 1998). However, the abiotic availability of strands of RNA that are long enough to function as a ribozyme or exhibit replicase activity is uncertain. There are also no indications that ribozymes with polymerase activity have ever existed since the only naturally occurring ribozymes do not perform polymerization reactions (Doudna and Cech, 2002; Freeland et al., 1999). The transition from ssRNA as the informational carrier to dsDNA is also mechanistically difficult since in order to maintain existing catalytic function, the concomitant evolution of a transcription system based on dsDNA is needed. Therefore, the fundamental molecular mechanisms that underlie the origin of life are still unknown.

The potential of RNA to perform as both an information carrier and as a catalytic molecule, is at the basis of the RNA world hypothesis. However, it is difficult to imagine ssRNA to have a dual function since the catalytic properties of RNA depend on the three-dimensional structure, while the informational capacity would require a simple linear structure that can be replicated (Taylor, 2005). The folding of an RNA molecule would prevent its own replication, while for replication a folded ribozyme would be necessary. Therefore, a dual function for the informational and the catalytic functions of ssRNA seem to be mutual exclusive on a biochemical mechanistic basis. From a system perspective, a dual function in a single entity would create a dependency that would reduce evolvability of the system, since each adaptation of one function could negatively affect the other function and prevent independent evolution. Thus, a scenario that is not dependent on a dual function of ssRNA could be a starting point for an alternative mechanisms for the origin of Life.

The 'dsRNA first' hypothesis

An independent evolution of both informational and catalytic functionalities of RNA would provide the necessary flexibility for the evolution of the genome. The potential for system evolvability is met in software development by using the design-by-contract methodology that views a system as a set of communicating modules whose interaction is based on precisely defined interfaces. Application of this design paradigm has recently led to alternative hypotheses for the origin of introns and the origin of the nucleus. Evolution based on design-by-contract implies that as long as existing interfaces stay intact,

independent evolution of the individual modules is possible. In case of the genome, we can discern two separate functional modules: the ability to store genetic information on one hand, and the ability to function as a catalyst on the other hand. In all organisms, the informational and catalytic entities in the genome are well-separated into respectively dsDNA and ssRNA, and can therefore be seen as the mechanistic implementation of these functional modules. Their relation or interface is strictly defined and conserved throughout the Tree of Life: one part of the double-stranded DNA represents the template for the transcription of ssRNA. Thus, the separation of the informational and catalytic characteristics of RNA by a defined interface allows in principle an independent evolution of both functions as long as this interface stays intact.

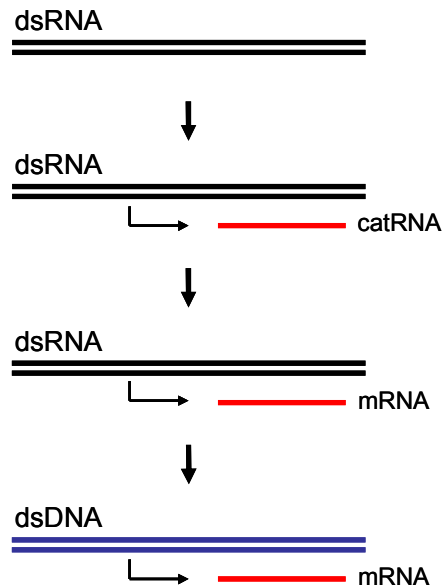


Fig. 1. The origin of genetic information carriers based on the conservation of existing interfaces. The separation of informational and catalytic properties of RNA can be accomplished when dsRNA evolved first as the informational molecule, and was followed later in evolution by ssRNA as the catalytic molecule. In this scenario, catalytic RNA is derived from initial dsRNA and at a later stage, this ssRNA can then act as the mRNA template for protein synthesis. A transition to dsDNA does not require a new kind of information carrier, but only the chemical adaptation of the existing one.

Since single-stranded catalytic RNA is derived from a double-stranded template, a straightforward explanation for the origin of catalytic RNA is when double-stranded RNA would also have preceded the catalytic single-stranded RNA in evolution. If this interface would be established first in evolution, it could have always remained intact during evolution. In this way, double-stranded RNA carried the informational function consistently from the start, while ssRNA always harboured the later-derived catalytic function (**Fig. 1**). This scenario keeps information flow through evolution intact and does not require a substantial change of interfaces during evolution. For instance, the dsRNA template could be changed later in evolution to a dsDNA interface without conceptually affecting transcription of ssRNA. Also, mRNA could be added as the template for protein translation without needing a substantial change in the existing carriers of genetic information, in contrast to a scenario where an existing ssRNA genome would be migrated to a dsRNA (dsDNA) genome. Thus, based on a simple engineering paradigm, it is proposed that dsRNA as the informational molecule was the first molecule to have evolved, and that catalytic ssRNA as the first ribozymes would be derived later in evolution.

A mechanism for the origin and evolution of the genome

From RNA oligomers to dsRNA

The 'dsRNA first' hypothesis about the origin of life states that the first functional step was the appearance of dsRNA before the development of catalytic RNA and thus implies that dsRNA appeared abiotically. Assuming that short strands of RNA formed by template-directed abiotic ligation were available in a prebiotic world (for discussion see Lazcano and Miller, 1996; Orgel, 1998), dsRNA can be formed by the hybridization and ligation of complementary sequences of oligonucleotides (**Fig. 2**). In this process, oligonucleotides could initially build up an interrupted double-stranded chain of RNA, which was followed by a non-enzymatic ligation reaction to form an uninterrupted double-stranded piece of RNA. This mechanism is similar to the template-directed, non-enzymatic ligation and amplification of oligonucleotides (Inoue and Orgel, 1983; Sievers and Von Kiedrowski, 1994; Doudna et al., 1991; Xu and Kool, 1999; Gao and Orgel, 2000; Chen et al., 1985; Sinclair et al., 1984) proposed to replicate an existing catalytic RNA molecule. Thus, the template-directed hybridization and extension of short oligonucleotides is a feasible approach for abiotic formation of longer strands of dsRNA.

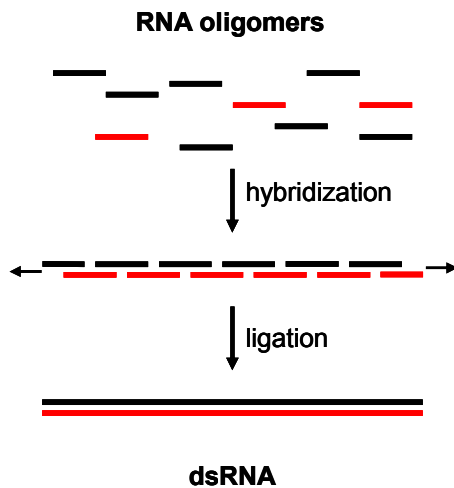


Fig. 2. Abiotic generation of dsRNA as a primitive information carrier. Based on the availability of oligoribonucleotides, the first step in the generation of dsRNA is initiated when small oligonucleotides stick together to form an interrupted double-stranded chain of RNA. Ligation of the oligonucleotides by a slow abiotic process leads to a tight dsRNA chain.

Replication by an abiotic chain reaction

The crucial step in the origin of life is the formation of a replicating system that will allow genetic information to be transduced and amplified. The replication of dsRNA can be based on the intrinsic properties of complementary strands of ribonucleotides to form a stable double-stranded helix below the melting temperature of dsRNA and to separate into individual strands at temperatures above its melting temperature (Lathe, 2003; Breivik, 2001). After temperature-induced strand separation, oligonucleotides can rehybridize to both individual strands upon lowering of the temperature to form new chains of interrupted dsRNA (Fig. 3). After non-enzymatic ligation of the interrupted strands (cf. Fig. 2), two new strands of dsRNA are formed. These newly formed dsRNA strands can then re-enter melting and rehybridization cycles, thereby amplifying the original strands of dsRNA. This process is similar to the polymerase chain reaction (PCR) used to amplify dsDNA, albeit with much slower polymerization time and could be driven by the diurnal cycle (see Discussion). The hybridization and extension of primer oligonucleotides to each other is frequently seen in PCR (e.g. Rychlik, 1995; Cawthon, 2002; Wege et al., 2003; Brownie et al., 1997) showing that thermal cycling in combination with ligation reaction can elongate as well as replicate oligonucleotides *in vitro*. Thus, using only non-catalytic short strands of ssRNA, dsRNA as an informational molecule can be formed and replicated abiotically.

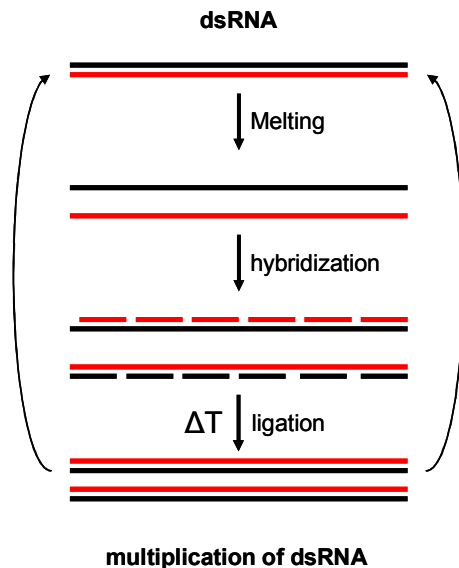


Fig. 3. Abiotic chain reaction to generate a pool of dsRNA. Melting of dsRNA by an increase in temperature will result in two separate daughter strands. Upon lowering of the temperature, oligonucleotides can hybridize to the individual strands that are subsequently abiotically ligated to form a new chain of dsRNA. This dsRNA can then reenter the melting-polymerization chain, leading to an exponential increase or replication of the initial dsRNA strand. This process is similar to the polymerase chain reaction that amplifies dsDNA.

An early transcription mechanism

The next step after the establishment of a pool of replicating dsRNA as a genetic information carrier is the subsequent generation of ssRNA that could function as a ribozyme (catalytic RNA; cf. Fig. 1). This generation of ssRNA from dsRNA can be accomplished by the partial melting of the double-stranded helix, followed by the hybridization of RNA

oligonucleotides to the resulting partially separated strand (Fig. 4). The hybridized oligonucleotides can then be connected by a slow abiotic ligation process, basically similar to the one that is proposed in figures 2 and 3 in the generation and replication of dsRNA. This ssRNA can also be elongated in subsequent cycles since the partial sequence will selectively rehybridize with its anti-sense RNA, leading to a full size 'transcript' in multiple ligation cycles. A subsequent release and folding of the hybridized RNA sequence from the template strand at higher temperatures would produce the first catalytic RNA (or later mRNA), a process that uses the same interface and is conceptually similar to *enzyme-assisted* transcription.

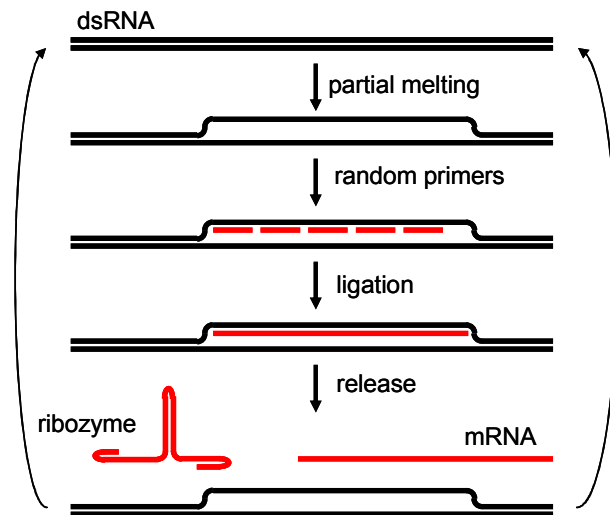


Fig. 4. Abiotic generation of the first catalytic RNA molecule. The generation of ssRNA as a catalytic molecule can be accomplished by the partial melting of a double-stranded RNA helix, followed by the hybridization of RNA oligonucleotides to the resulting ssRNA strand upon lowering of the temperature. The hybridized oligonucleotides are then be annealed by a slow abiotic ligation process, similar to the one that is proposed in Figure 2 in the generation of dsRNA. The partial melting of the dsRNA, the rehybridization, and the release of the ssRNA can be accomplished by an oscillation in temperature.

A change in temperature and/or salinity may cause a partial melting in regions of the dsRNA where the local melting temperature is lower due to the base composition. This partial melting or 'breathing' is a well-described phenomenon in dsDNA that occurs when thermal fluctuation opens up part of the dsDNA (passive opening) and allows components of the transcription machinery to bind the exposed single-stranded DNA (Betterton and Jülicher, 2005; Blake and Delcourt, 1998). Regions of RNA with specific nucleotide sequences (e.g. AU-rich) that have low local melting temperatures are more subject to partial melting and these specific regions could have represented early genes. Partial transcripts that rehybridize with partially melted RNA may also prevent the helix to close and thereby enhance their own transcription.

From a dsRNA to a dsDNA world

The advent of protein generation (translation) can speed up evolution by replacing existing abiotic replication and transcription processes by more efficient protein enzymes, for instance RNA polymerases. Based on the presence of existing dsRNA, in only a few gradual changes in an existing RdRp, the transition from a dsRNA to a dsDNA world can be made (Dworkin et al., 2003). This transition is conceptually simple because it can be made by substituting the ribonucleotides building blocks of RNA with the deoxynucleotides of DNA (cf. Fig. 1). The insertion of deoxynucleotides by turning an existing RNA-dependent RNA polymerase (RdRp) into an RNA-dependent DNA polymerase RdDp) would effectively create a DNA-RNA hybrid, while the concomitant evolution to a DNA-dependent DNA polymerase (DdDp) would create dsDNA (Fig. 5A). The existing transcription process would only need a change in the template recognition site of an RdRp to a DdRp polymerase (Fig. 5B). Substrate specificity can be influenced by subtle modifications to a generic polymerase module (Joyce, 1997) and most DNA polymerases are able to incorporate rNTPs as a substrate instead of dNTPs (Astatke et al., 1998, Gao et al., 1997; Bonnin et al., 1999, Ruiz et al., 2003). Also, template recognition of polymerases is not very specific and can be changed by minor mutations (Siegel et al., 1999; Fisher et al., 2003; Patel and Loeb, 2001, Boulé et al., 2001). This would not have affected existing replication mechanism or involved the *de novo* development of a transcription machinery.

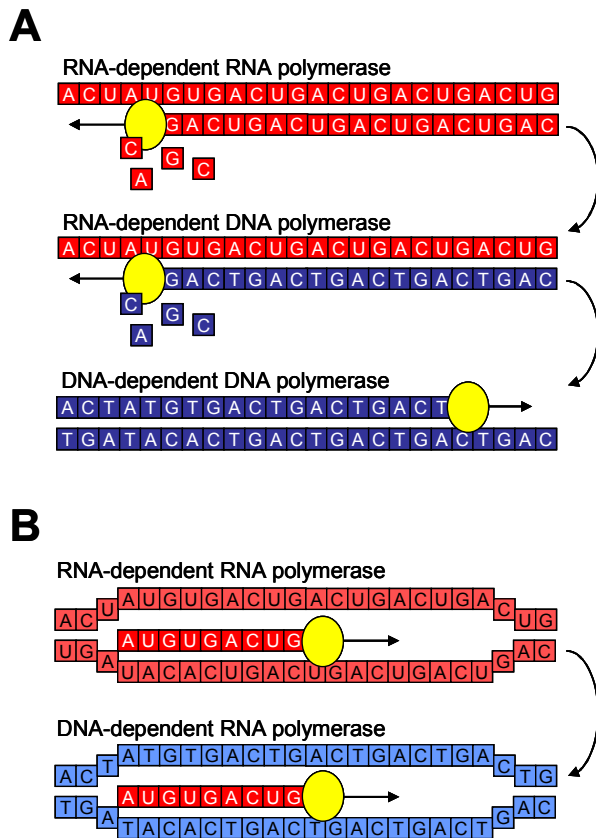


Fig. 5. Gradual transition from an RNA to a DNA world. **A.** The transition from dsRNA to dsDNA can be accomplished by substituting RNA nucleotides for DNA nucleotides. This would require a switch in substrate-specificity from a RNA-dependent RNA polymerase to an RNA-dependent DNA polymerase, followed or accompanied by a mutation in the template specificity to a DNA-dependent DNA polymerase. **B.** The transcription of RNA from dsDNA is conceptually similar to transcription from dsRNA and this transition requires only a change in template recognition, i.e. from a RNA-dependent to a DNA-dependent RNA polymerase.

Discussion

Thermally-cycling microenvironments

Some form of compartmentalization in, for instance, liposomes or porous rock (Monnard and Deamer, 2001; Russell and Hall, 1997; Martin and Russell, 2002) is a necessary prerequisite for containing the early constituents of life. The first oligomers could have formed in protocells when activated monomers react with monomers or short oligomers bound to the surface of a catalytic mineral such as montmorillonite (Ferris et al., 1996; Ferris, 1999). The presented gradual dsRNA first hypothesis for the origin of life may have started when short strands of complementary oligonucleotides (see Orgel, 1998; Ferris, 1999) form in populations of abiotic microenvironments, for instance the pores of porous rock. Later in evolution, the evolution of lipid-generating enzymes could generate a cell membrane which could replace the existing abiotic microenvironments (de Roos, 2006).

The *proposed* melting and rehybridization cycles underlying the dsRNA-first hypothesis can be driven in a prebiotic environment by a diurnal cycle that amplifies and replicates populations of dsRNA. The perpetual night/day temperature cycles can in this view considered to be the driving force for the emergence of Life, creating an early potential for diversity and selection with each replication cycle. The thermal cycling PCR can in principle also occur in a steady temperature gradient where convection will cycle the DNA in and out of the higher temperature spot (Braun et al., 2003; Krishnan et al., 2002), widening the environments for an origin of life based on thermal cycling. Melting and hybridization steps are in principle also possible by tidal cycling (Lathe, 2003) in combination with drying and increasing salt concentration, since increasing ionic strength also favors melting.

Evolutionary drive towards formation of catalytic RNA from proto-genes

The tendency of ribonucleotides to hybridize and ligate to form dsRNA will cause the preferential formation and accumulation of long strands of dsRNA in the first thermally cycling proto-cells. A selection for the 3-5 orientation of the double helix could be caused by the higher resistance to hydrolysis when compared to the 2-5 form (Usher and McHale, 1976). The abiotic transcription process would be specifically enhanced for proto-genes that generate a folded RNA. Since

folded RNA's are less likely to rehybridize with its template in the abiotic transcription process, they avoid the product inhibition observed in other replicating systems (Sievers and Von Kiedrowski, 1994). Ribozymes and catalytic RNA are characterized by their folding into a three-dimensional structure like hairpin loops, and in combination with random mutation, a selection can take place for single-stranded RNA with catalytic abilities. Selection of protocells in which ssRNA is generated that can specifically aid in the replication process may lead to selection for autocatalytic populations of dsRNA and may be considered to be proto-genomes. The emergence of ribozymes may also aid in the generation of genetic diversity by catalyzing splicing and ligation of RNA strands (cf. the role of introns; de Roos, 2005).

A gradual mechanistic scenario for the origin of Life

The dsRNA first hypothesis proposes a sequence of functional events for the evolution of the genome that is derived from engineering principles. This proposed scenario could be translated into a gradual sequence of biochemical, mechanistic events by using the basic hybridization reaction between complementary ribonucleotides and the abiotic ligation reaction as a basis for the origin of Life. The dsRNA first hypothesis can be separated into distinct mechanistic and functional steps: a) the abiotic formation of dsRNA as a starting point for Life, b) the replication of this dsRNA in order to conserve and multiply the potential informational carrier, c) the formation of catalytic ssRNA from specific proto-gene regions of dsRNA, d) extension of functionality by protein translation based on a single-stranded RNA template (mRNA), and e) the transition from dsRNA to dsDNA. Although the exact timing of the biochemical events and driving forces that took place in early genome evolution remain speculative, the proposed sequence of events could not only explain the origin of Life, but also provides a testable hypothesis for *in vitro* biochemical evolution. Moreover, the proposed evolution of the genome may provide an example for the generation of self-evolving systems *in silico*.

A design framework for evolution

Design-by-contract (Meyer, 1997) forms a simple straightforward framework to design complex systems that need to be extensible and robust. Evolutionary steps can be modelled on this engineering paradigm by defining evolution as an expanding system of functionalities that are connected by well-defined and constant interfaces. The effect of designing interfaces across modules is a reduction of the interdependencies across modules or components and a reduction of the risk that changes within one module will create unanticipated changes in other modules. By identifying the functional modules and their conserved interfaces in evolution, it becomes possible to reconstruct the mechanistic scenarios underlying evolution. Here, an analysis of the interfaces for transcription and translation defined dsRNA as the logical first step towards an expanding genome. This approach led to a relative simple scenario for the origin of Life that makes a gradual scenario for genome evolution feasible. The same approach has recently also led to new scenario's for the origin of introns (de Roos, 2005) and the origin of the eukaryotic cell (de Roos, 2006) and shows the explanatory power of modelling evolution on design-by-contract and viewing Life as a self-evolving molecular machine.

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