

Difficulties of this type, and a number of other problems, caused Gerald Joyce and Leslie Orgel (2006) to declare that the abiotic formation of RNA would constitute a “near miracle”. (Many other chemists, myself included, agree with this assessment). If we reject the idea that RNA, and other information-rich biopolymers were present at the start of life, then we arrive at an alternative, satisfactory solution for their origin. RNA first appeared through natural selection in living organisms, as the result of an extensive series of events, each of which had its own justification.

If we accept this argument, then we must conclude that the earliest forms of life functioned through the activities of sets of smaller, abiotically available molecules. To understand the origin of life, we must understand and if possible model these processes.

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Linking the RNA World to Modern Life: The Proto-Ribosome Conception

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In order to tackle the plausibility of the ‘RNA world’ hypothesis we aimed at scrutinizing an ancient self-folded RNA entity that functioned in the RNA world and evolved into a simple machine capable of catalyzing chemical reactions, including the formation of peptide bonds. By exploiting structural, biochemical, computational and modeling experiments, the remnant of this machine was identified within the contemporary ribosome (Baram and Yonath 2005; Agmon et al. 2006; Agmon et al. 2009; Davidovich et al. 2009 Yonath 2009).

The ribosome is the universal multi-component macromolecular cellular assembly that decodes the genetic information and efficiently elongates nascent polypeptide chains under the mild conditions of the modern life. The contemporary ribosomes are ribonucleoprotein assemblies, with molecular weights of 2.5 and 4MDa (for prokaryotes and eukaryotes, respectively). Despite this significant differences in their sizes, the core functional regions of ribosomes from all kingdoms of life exhibit remarkable conservation. Among them is a universal symmetrical 'pocket-like' sub-structure, an extraordinary feature in the otherwise asymmetric ribosome. It is composed of 180 ribosomal RNA (rRNA) nucleotides (Bashan et al. 2003 MC; Agmon et al. 2005), and hosts the peptidyl transferase center (PTC) namely the site of peptide bond formation. This symmetrical region provides the framework for the positioning of the ribosomal substrates in favorable stereochemistry for peptide bond formation and for substrate-mediated catalysis (Bashan 2003 et al. MC; Gregory et al. 2004; Bieling et al. 2006; Weinger et al. 2007; Bashan and Yonath 2008). Furthermore, by encircling the PTC the architecture of this region confines the void required for the motions involved in tRNA 3' end translocation, required for the successive peptide bond formations, thus enabling the ribosome polymerase activity (Bashan et al. 2003; Agmon et al. 2005).

The ribosomal symmetrical region seems to be preserved throughout evolution (Agmon et al. 2006; Agmon et al. 2009; Davidovich et al. 2009; Yonath 2009) and the fold of each of its halves resembles the main building block of "ancient" as well as "modern" functional RNA molecules of comparable size (e.g. gene regulators, riboswitches, ribozymes catalyzing the phosphodiester cleavage, RNA processors etc), hence suggesting that it could have existed in the RNA world as a self folded autonomous entity. This entity could have functioned as an apparatus catalyzing various reactions involved in RNA metabolism, as well as peptide bond formation and non-coded oligopeptides elongation.

Support for the existence of RNA entity capable of self replication, folding and dimerization are the recent non-enzymatic synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions (Powner et al. 2009; Szostak 2009) and the demonstration that RNA oligomers can be obtained non-enzymatically from activated RNA precursors (Pino et al. 2008; Krzyaniak et al. 1994). The dimerization in a symmetrical manner of self folded motifs of identical, similar or different sequences, may have occurred spontaneously, resulting occasionally in 'pocket-like' structures capable of hosting spontaneously produced amino-acids conjugated with single or short oligonucleotides, (Ilangasekare et al. 1995; Giel-Pietraszuk et al. 2006; Lehmann et al. 2008) serving as substrates for peptide bond formation.

The more stable constructs of these 'pocket-like' molecular dimers might have survived under various environmental conditions. Among them, those that would accommodate suitable substrates at the appropriate stereochemistry enabling peptide bond formation have been evolutionarily favored. As it is assumed that in the prebiotic era RNA chains could self replicate (Eigen 1993; Smith and Szathmary 1995; Lincoln and Joyce 2009; Woese 2001; Yarus 2002), it is conceivable that phenotypes with favorable properties could have been synthesized in many copies. It is likely that some of these phenotypes were originated by fusion of two different or duplication of two identical sequences, resembling gene elongation events (Fani and Fondi 2009).

Based on the high conservation of the ribosomal region assigned as the proto-ribosome and on its capability to provide all of the architectural elements required for

amino acid oligomerization, we assumed that it existed in the RNA world and functioned in a fashion similar its precedent within to the contemporary ribosome. The hypothesis of a self assembled ribosomal active site, which is still implanted in the internal core of the modern ribosome, triggered biochemical experiments aimed at revealing the tendency for self folding and dimerization of RNA chains. These yielded biochemical evidence supporting the existence of a dimeric proto-ribosome, and provided hints for a feasible pathway for acquiring the structural elements necessary for coded amino acid polymerization. Hence shedding light on the emergence of the contemporary genetic translation apparatus from rather short RNA oligomers (Fig. 1).

We found that some, albeit not all, RNA chains with sequences resembling those observed in the current ribosome, are capable of forming dimers that may adopt a ‘pocket-like’ structure (Davidovich et al. 2009). Furthermore by site-directed mutagenesis we showed that the tendency for dimerization, a prerequisite for obtaining the catalytic centre, is linked to the fold of the proto-ribosome two components, thus indicating that functional selection at the molecular level existed already in the prebiotic era. Consistently, it is conceivable that ‘pocket-like’ RNA entities were assembled spontaneously from a pool of RNA chains.

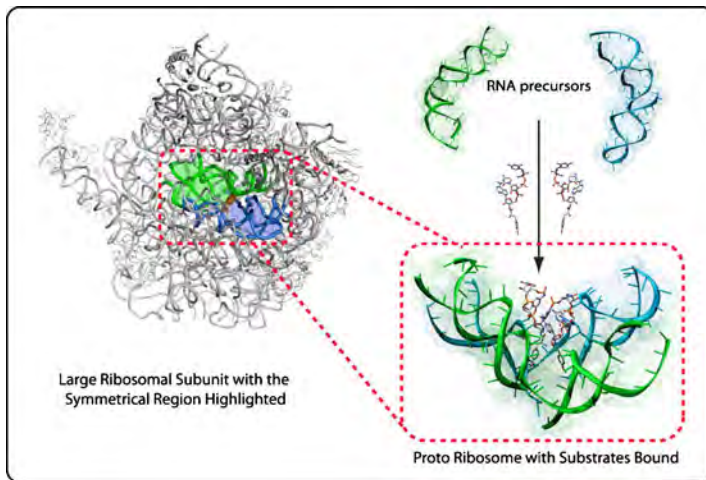


Fig. 1 Left: the symmetrical region at the heart of the large ribosomal unit. Its two halves are colored in blue and green and actual peptide bond formation site is shown in red.

Right: Top: the precursors of the proto-ribosomes in their assumed conformation Bottom: the pocket-like entity resulting from the dimerization of the two precursors

Among the products of these early amino acid elongation processes, those molecular entities possessing central, albeit primitive catalytic and/or synthetic properties, became the templates for enhanced production (see Belousoff et al. this issue), survived evolution pressures, and underwent natural selection. Among the key tasks performed by the initial oligopeptides is stabilizing the proto-ribosome and/or other components confined in its surrounding, within assemblies that could evolve into “proto-cells”. As it is likely that

subsequently the proto-ribosomes underwent optimization from non-genetic peptide bond formation towards performing genetically driven translation, it is conceivable that the ancient proto-ribosome in its functionally-optimized version is still embedded in the core of the modern ribosome, and that the symmetrical region of the modern ribosome originated from the proto-ribosome.

In short, here we present structural tools for investigating possible pathways in the evolution of modern life and approaching key questions, such as: Did the ancient translation apparatus survive selection pressure? Does its relic reside within the modern ribosome? What was the evolution conduit leading to its successive optimization?

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A Model for the Modular Evolution of RNA Addressing Open Questions on the Origin of Life

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A main unsolved problem in the RNA world scenario for the origin of life is how a template-dependent RNA polymerase ribozyme emerged from short RNA oligomers generated by random polymerization of ribonucleotides (Joyce and Orgel 2006). Current estimates establish a minimum size about 165 nt long for such a ribozyme (Johnston et al. 2001), a length three to four times that of the longest RNA oligomers obtained by random polymerization on clay mineral surfaces (Huang and Ferris 2003, 2006). To overcome this gap, we have developed a stepwise model of ligation-based, modular evolution of RNA (Briones et al. 2009) whose main conceptual steps are summarized in Figure 1. This scenario has two main advantages with respect to previous hypotheses put forward for the origin of the RNA world: i) short RNA