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In vitro selection of halo-thermophilic RNA reveals two families of resistant RNA ☆

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Abstract

The "RNA world" hypothesis proposes that early in the evolution of life, RNA was responsible both for the storage and transfer of genetic information and for the catalysis of biochemical reactions. One of the problems of the hypothesis is that RNA is known to be temperature sensitive. Nevertheless, different types of sequences with a thermostable phenotype may exist. In order to test this possibility, we applied an in vitro evolution method (SELEX) to isolate RNA molecules that are resistant at high temperatures (80 °C for 65 h) and high salt concentrations (2 M NaCl). The sequences of the resulting cloned halo-thermophilic RNAs can be grouped in two families (I and II) possessing very different thermal and chemical stabilities and very different secondary structures. The selected RNA molecules illustrate two different possibilities leading to thermal resistance which may be related to primitive conditions. We propose that members of family I constitute a good means of storing sequence information while members of family II are less efficient but replicate faster in early steps of the SELEX. These selected RNA behaviors may be related to primitive conditions and could allow to define limits for survival, and demonstrate that what is at stake for RNA molecules, as for living organisms, is survival and reproduction.

Keywords: RNA world; High temperature; High salt concentration; RNA stability; Amplification; Early evolution; Origins of life

1. Introduction

An important issue in the problem of the origins of life is whether or not an RNA world may be compatible with extreme primordial conditions. This scenario of evolution postulates that an ancestral molecular world is common to all present forms of life; the functional properties of nucleic acids and proteins as we see them today would have been carried out by molecules of ribonucleic acids. RNAs occupy a pivotal role in the cell metabolism of all living organisms and several biochemical observations resulting from the study of contemporary metabolism should be stressed. For example, throughout its life cycle, the cell produces deoxyribonucleotides required for the synthesis of DNA that are derived from ribonucleotides. Thymine, a base specific of DNA, is obtained by transformation (methylation) of uracil, a base specific of RNA, and RNAs serve as obligatory primers during DNA synthesis. Furthermore, the current characterization of catalytic RNA molecules strengthens the RNA world hypothesis (Gilbert, 1986; Gesteland et al., 1999; Joyce, 2002) and is an additional argument in favor of the presence during evolution of RNAs before DNA.

Several investigations have corroborated the possible role of RNA in early life (Woese, 1967; Crick, 1968; Orgel, 1968),

Abbreviations: SELEX, Systematic Evolution of Ligands by Exponential enrichment; PCR, Polymerase Chain Reaction; dNTP, 2′-deoxyribonucleotide 5′-triphosphate; rNTP, ribonucleotide 5′-triphosphate; Tris, tris(hydroxymethyl) aminomethane; EDTA, ethylene diamine tetracetate; DTT, dithiothreitol; M-MLV, moloney murine leukemia virus; cDNA, DNA complementary to RNA; CD, circular dichroisme; NIH, National Institutes of Health; bp, base pairs; RT-PCR, reverse transcription-polymerase chain reaction; Tm, melting temperature.

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including data on template-directed synthesis of macromolecules (Joyce and Orgel, 1999), oligomerisation on mineral surfaces (Ferris et al., 1996), and possible transitions from an alternative genetic system to RNA (Schmidt et al., 1997; Kozlov et al., 1999; Chaput et al., 2003). DNA has been considered as modified RNA, better suited for the conservation of genetic information. This genetic privilege would constitute a logical step in an evolutionary process during which other molecules could have preceded RNA and transmitted genetic information.

From a fundamental point of view, it is interesting to study the behavior of nucleic acids under extreme conditions (Tobé et al., 2005). It is generally assumed that the upper temperature limit for life is related to the instability of essential key molecules such as nucleic acids (Marguet and Forterre, 1994). Many RNA molecules including members of a growing list of artificially selected aptamers are single-stranded, and much more labile than single-stranded DNA. RNA is thermosensitive creating a problem if early life was thermophilic as is assumed in some scenarios (Wächtershäuser, 1992), compatible with some views on the last common ancestor (Di Giulio, 2000, 2003a,b; Schwartzman and Lineweaver, 2004).

In addition, there are indications that primitive oceans on earth were highly saline (Knauth, 1998), and early life has been discussed in terms of hypersaline conditions (Dundas, 1998). Salt and water are tightly linked: salt is abundant on Mars, and on Europa one of the satellites of Jupiter (Kargel, 1998). Finally, very ancient salt-tolerant bacteria have been extracted from brine inclusions in salt crystals (Vreeland et al., 2000). These arguments are all in favor of an extreme saline primordial environment.

The fragility of RNA structures and activity however, is the subject of controversy and requires direct experimentation. In vitro selection has been used for creating and identifying molecular interactions. It has been successfully used to isolate and characterize thermodynamically stable tri- and tetraloop RNA hairpins (Bevilacqua and Bevilacqua, 1998; Shu and Bevilacqua, 1999; Proctor et al., 2002). A selection has been devised to select RNAs based on their local folding stability. A large RNA molecule with 19 randomized nucleotides shows dependence upon tetraloop identity and on mutations in the nonrandomized region of the RNA (Juneau and Cech, 1999, Guo and Cech, 2002). Regarding the abundance of halite minerals in the early environment, it is important to consider the role of high salt concentrations on macromolecules. We have already shown the effect of high salt concentrations on the structural integrity of tRNAs and on maintaining residual specific charging capacity after heat treatment of the tRNAs for 30 h at 82 °C (Tehei et al., 2002). These studies have provided support for the importance of salt in the protection of macromolecules against thermal degradation allowing their activity to be maintained. In order to learn more about the biochemical properties of RNA molecules, we studied the stability of such molecules selected in vitro at high temperatures in the presence of salt using the SELEX (systematic evolution of ligands by exponential enrichment) method (Tuerk and Gold, 1990). Our results, which brought to light two families of thermal resistant RNA molecules could also be useful in the search for traces of life in ancient sediments and in planetary exploration.

2. Materials and methods

2.1. Preparation of the starting RNA pool

Single-stranded DNA template and primers (Marshall and Ellington, 2000; Meli et al., 2002, 2003) were chemically synthesized (Proligo and MWG-Biotech). The sequence of the forward primer 1 (P1) is 5′-**GGTAATACGACTCACTA-TA**GGGAGATACCAGCTTATTCAATT-3′(T7 promoter sequence in bold), and of the reverse primer 2 (P2) is 5′-AGATTGCACTTACTATCT-3′. The 86 nt-long template (T) 5′-ATACCAGCTTATTCAATT-$(N)_{50}$-AGATAGTAAGTG-CAATCT-3′ consists in a 50 nt-long random sequence where *N* stands for any nucleotide, flanked by two constant regions of 18 nt each for PCR amplification.

A 2 ml PCR (Invitrogen) reaction mixture containing 1 μM of each primer P1 and P2, 0.085 μM template T (10^{14} random molecules), 0.2 mM dNTPs, 20 mM Tris–HCl pH 8.4, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.025 u/μl of Taq DNA polymerase, was treated using the following cycling parameters: denaturation at 94 °C for 30 s, annealing at 40 °C for 2 min and elongation at 68 °C for 2 min.

After PCR, the starting random dsDNA pool was ethanol precipitated and submitted to in vitro transcription: the 1.65 ml reaction mixture contained 2 mM each rNTP, 40 mM Tris–HCl pH 7.9, 2 mM spermidine, 6 mM $MgCl_2$, 10 mM NaCl and 10 mM DTT (Fermentas buffer), 0.02 mg/ml of dsDNA pool and 0.6 u/μl T7 RNA polymerase (Fermentas). After overnight incubation at 37 °C, it was ethanol-precipitated and the DNA template degraded by DNase 1 (RQI RNase-free DNase, Promega). The RNA was deproteinized, ethanol-precipitated, and purified on a 6% denaturing polyacrylamide gel (19/1 acrylamide/bisacrylamide, Tris–borate–EDTA buffer pH 8.3 and 7 M urea). The RNA was eluted from the gel, treated with phenol, phenol/chloroform/isoamyl alcohol, twice

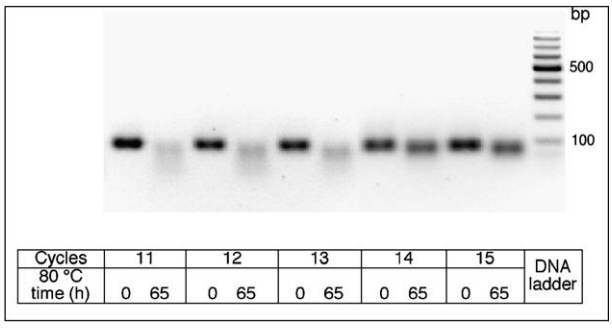

Fig. 1. Selection evolution during cycles 11 to 15 of the SELEX procedure. Analysis by native agarose gel electrophoresis of RNA integrity, at time 0 and after 65 h at 80 °C in 2 M NaCl.