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Minireview

Omnipotent RNA

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Abstract The capability of polyribonucleotide chains to form unique, compactly folded structures is considered the basis for diverse non-genetic functions of RNA, including the function of recognition of various ligands and the catalytic function. Together with well-known genetic functions of RNA – coding and complementary replication – this has led to the concept of the functional omnipotence of RNA and the hypothesis that an ancient RNA world supposedly preceded the contemporary DNA–RNA–protein life. It is proposed that the Woese universal precursor in the ancient RNA world could be a cell-free community of mixed RNA colonies growing and multiplying on solid surfaces.

Key words: Non-coding RNA; RNA folding; RNA recombination; RNA world; RNA colony; Universal precursor

1. Historical introduction: Discovery of non-coding RNA

By the middle of the past century E. Chargaff established the fact of species specificity of the base composition of DNA: it was demonstrated that ratios of the four sorts of DNA monomers – A, G, C and T – can differ in different taxa of living beings [1,2]. This fact corresponded to the genetic role of DNA already accepted by many scientists at that time. After T. Caspersson and J. Brachet [3,4], the role of the other type of nucleic acids – RNA – was assumed to serve protein synthesis in the cytoplasm. The model of DNA structure proposed by J.D. Watson and F.H.C. Crick 50 years ago [5] immediately suggested the mechanism of DNA reduplication and also the possibility of replication of RNA on DNA [6]. Soon after the so-called central dogma of molecular biology was proclaimed: DNA → RNA → protein. Thus, RNA was presumed to function as the genetic intermediary between DNA and proteins that copies DNA and serves as a template for protein synthesis.

According to the above-mentioned conception, the RNA base composition (the ratio of the four sorts of monomers) must reflect the variations of the base composition of DNA. A great extent of variations in DNA composition was revealed among bacteria, the (G+C)/(A+T) ratio being from 2.7 to 0.45. Unexpectedly, the RNA base composition was found to be relatively conserved, the (G+C)/(A+U) ratio varying only from 1.05 to 1.45 [7,8]. That was a confusing result: "The evidence presented there showed that our ideas were in some important respects too simple" [9]. In any case, the results suggested a significant portion of total RNA of the cell to be non-genetic RNA.

At the same time, the statistical analysis of the above data indicated that there was a positive correlation, though with a low regression, of the base composition of RNA with that of DNA [8]. These results were interpreted in such a way that the major part of cellular RNA is similar in different species (evolutionarily conservative RNA), and on this background there exists a small fraction of species-specific, DNA-like RNA. Somewhat earlier, the formation of DNA-like RNA was demonstrated during phage infection of bacterial cells: injection of phage DNA into the cell induced the synthesis of RNA similar to phage DNA in base composition [10]. The subsequent comparative analysis of base compositions of DNA and RNA [8] first indicated that the fraction of DNA-like RNA is a normal component of common, uninfected cells where it may fulfill the function of the transfer of genetic information from cellular DNA to determine the synthesis of cellular proteins. Later this fraction of RNA was called messenger RNA (mRNA) [11].

On the other hand, the discovery of the non-DNA-like, presumably non-genetic RNA stimulated further investigations of RNA functions. Soon after it became clear that the predominant mass of cellular RNA is the constituent of ribosomes, i.e. represents ribosomal RNA. It was proved that ribosomes and ribosomal RNA themselves do not carry genetic programs for protein synthesis [12–14]. Ribosomes were shown to form a universal, non-specific protein-synthesizing apparatus that must be programmed by mRNA to make gene-specific proteins. All the following studies of ribosomal RNA confirmed that this is an evolutionarily highly conservative constituent having no coding functions in living beings.

2. Compact self-folding of RNA chains

Studies on physical chemical properties of isolated high-polymer RNAs, including ribosomal RNA, in solution made during 1958–1962 (reviewed in [15,16]) had led to the conclusion that they are capable of self-folding into compact particles with both near-range and long-range intrachain interactions. In other words, RNA was proposed to form both a secondary structure, mainly as a set of short double-helical regions made by antiparallel complementary pairing of adjacent sections of the chain, and a tertiary structure acquired

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due to intrachain long-range complementary cross-links and interhelical interactions. The formation of compact structures by ribosomal RNAs was further confirmed in subsequent electron microscopy and neutron scattering studies. The main original result of these studies was the demonstration of the fact that under proper conditions the isolated ribosomal RNAs are capable of forming compact particles specific in their shape, depending on RNA species [17,18]. Thus, the ribosomal 16S RNA at high Mg^{2+} concentrations was shown to self-fold into uniform Y-like particles resembling 30S ribosomal subunits in their contour and dimensions, whereas the 23S RNA in the presence of spermidine at high Mg^{2+} was visualized as hemi-spherical particles similar to 50S ribosomal subunits but with somewhat reduced three protuberances. From all this, the conclusion on the capability of RNA to acquire compactly folded, unique conformations was drawn. Two specifically self-folded high-polymer RNAs (16S and 23S ribosomal RNAs) were proposed to form compact structural cores of the two ribosomal subunits (30S and 50S subunits, respectively).

Recently the structures of the ribosomal subunits and the whole bacterial ribosome were determined using X-ray crystallography with a resolution from 5.5 Å to 2.4 Å depending on the object [19–22]. In addition to rich information on detailed structure of the ribosomal particles, the results obtained fully confirmed the previous proposal that the morphology of the ribosomal subunits is determined by their compactly folded high-polymer RNAs. As is becoming clear, it is the specifically self-folded ribosomal RNAs and their interactions that determine the structural peculiarities and molecular mechanisms of the ribosome as a molecular machine.

3. Specific recognition of ligands by RNA folds

The capability of RNAs to form unique three-dimensional structures provides the basis for their specific interactions with other molecules, including both macromolecules and small ligands. Particular spatial patterns, sufficiently rigid, can arise on the surfaces of compactly folded RNAs. Thus, the function of selective molecular recognition must be assumed for RNA folds, similarly to the recognition of ligands by globular proteins.

The well-known recognition of oligonucleotide sequences due to Watson–Crick complementary interactions between exposed RNA chain sections is limited by the acts of RNA-to-RNA binding or communication. The recognition of various types of ligands by RNA compact folds is a novel theme that may be of great importance for comprehension of cell life and the origin of life. E. Cundliffe seems to be the first who lucidly proclaimed and substantiated the idea on the capability of structured regions of ribosomal RNA to recognize and bind small ligands of non-nucleic acid nature [23]. He communicated experimental evidence in favor of selective interaction of specific portions of ribosomal RNA, rather than ribosomal proteins, with several ribosome-aimed antibiotics, such as thiostrepton, erythromycin and aminoglycosides. Soon support came from the experiments on protection of ribosomal RNA bases from attack by chemical probes in complexes of ribosomes with aminoglycosides [24] and other antibiotics [25]. In about a decade direct structural studies proved the fact of the formation of a specific complex between an aminoglycoside antibiotic and an RNA structured element in the vicinity of the A site of the 16S ribosomal RNA [26] (see also the review [27]).

The widest potentialities of RNA to recognize other molecules and interact with them were definitively proved due to the invention of aptamers [28] – relatively small synthetic RNAs obtained by procedures of in vitro selection and 'test-tube evolution', such as SELEX [29] (see also [28,30]). It was found possible to select and amplify RNA molecules capable of specifically binding with any sort of other molecules, from low-molecular-weight organic compounds to various individual peptides and proteins (reviewed in [27,31]). Typically the RNA chain in simple aptamers is folded on itself into an imperfect (distorted) double helix with a specific recognizing pocket in the region of the helix defect. Expanding studies and applications of aptamers clearly demonstrate that RNA does possess the function of specific molecular recognition of a variety of ligands, analogously to proteins.

4. Catalytic functions and spontaneous recombinations of RNA

In the beginning of the 1980s the existence of RNA structures capable of catalyzing their own processing was discovered [32]. Independently it was found that the RNA moiety of RNase P, an enzyme performing the processing of tRNA precursors, is its catalytic subunit [33]. In both cases the catalytic RNAs were shown to form close-packed cores with well-developed secondary and tertiary structures [34,35]. By analogy with enzymes, the catalytic RNAs were called ribozymes. Later a series of the so-called small ribozymes, such as 'hammerheads' and 'hairpins', were found in nature (reviewed in [36]) and also created artificially by the in vitro selection/evolution technologies (see [31,34].

The natural ribozymes of the above-mentioned types are all involved in RNA processing reactions [34]. At the same time, artificially selected ribozymes demonstrate much wider catalytic potentialities of RNA molecules, including alkylation of a nucleoside, synthesis of aminoacyl adenylates (mixed anhydrides) from amino acids and ATP, aminoacylation of nucleotides and tRNA, amide (peptide) bond formation between amino acids, transpeptidation, and even carbon–carbon bond formation (reviewed in [31,34,37,38]). Also there is an important addition to the natural ribozyme scenery: a compactly folded domain of the ribosomal 23S RNA appears to be responsible for the peptidyl transferase activity of the ribosome, i.e. it seems to be a natural ribozyme catalyzing transpeptidation reaction during translation [39,40]. Among in vitro selected ribozymes, those that catalyze the ligation of RNA chains [41] and the polymerization of ribonucleotides from nucleoside triphosphates on an RNA template [42] deserve special attention (see Section 5).

In connection with the above, the recent discovery of spontaneous rearrangements and recombinations of sequence-nonspecific RNAs in solution [43] may be considered equally important. According to the experimental evidence presented, RNA chains of most diverse sequences can recombine at a rate of 10^{-9} h^{-1} per site, and the reaction is Mg^{2+}-dependent but does not involve free 3′-hydroxyls. The reaction seems to take place within complexes formed by base-paired sections of RNA molecules and can proceed both *in trans* (recombinations) and *in cis* (structure rearrangements). As follows from the strength of all the results, it is not due to cryptic ribozyme structures that might be formed by some RNAs, but is an

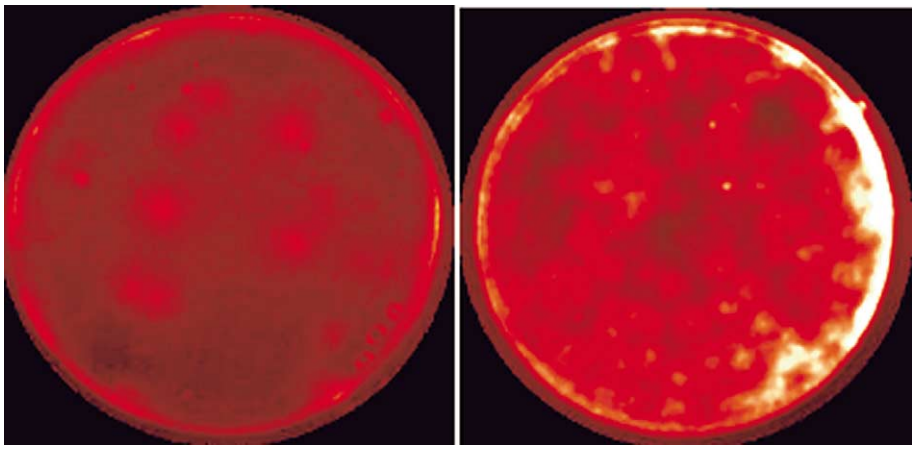

Fig. 1. RNA colonies on agarose gel [50]. Left-hand panel: Growth of RNA colonies in a covered Petri dish during 1 h at 25°C. Right-hand panel: Growth of RNA colonies in an open Petri dish during 1 h at 25°C. Qβ replicase (RNA-dependent RNA polymerase) and ribonucleoside triphosphates were present in the agarose gel. Stained with ethidium bromide. (Courtesy of A.B. Chetverin.)

intrinsic chemical property of polyribonucleotides. This capability of RNA could be antecedent to the ribozyme functions. A vast diversity of RNA species could be generated by this pre-ribozymic mechanism during the time left by Nature.

5. Genetic functions of RNA and the ancient RNA world

> "Biologists considered it as a primitive formation – a sort of gigantic being, a single enormous fluid cell (which they called 'prebiological'), surrounding the globe with a colloidal envelope..."
>
> Stanislaw Lem, *Solaris*

Thus, RNA molecules prove to be capable of doing principally all that proteins can do, such as self-folding into specific three-dimensional structures and determining shape formation of biological particles, recognizing other macromolecules and small ligands with high precision and selectively binding them, and performing catalysis of covalent reactions between recognized molecules. But proteins cannot replicate themselves: there do not exist molecular mechanisms for self-reproduction of proteins, except the mechanism via RNA. At the same time, RNA has all the structural prerequisites necessary for replication of its own structure. Indeed, RNA genomes are widely spread among viruses, and their replication in infected cells is known to proceed via complementary RNA chains. It is likely that the RNA-directed RNA replication can in some cases also take place in normal cells; anyway, functioning and formation of newly discovered small RNAs of the siRNA and miRNA classes involved in translational regulation and/or antiviral protection require their self-dependent replication in the cell [44].

Hence, RNA appears to be the most self-sufficient substance of the living matter: it is principally capable of performing all or almost all functions that are characteristic of proteins, and at the same time it can serve as a genetic material with replicative and coding functions, like DNA. From this, the hypothesis on the ancient RNA world, which could precede the contemporary DNA–RNA–protein life, has arisen [45–48]. According to the hypothesis, there once existed neither proteins nor DNA, but just ensembles of replicating RNA molecules. As it was unlikely that their 'cultivation medium' contained abiogenic substrates for RNA synthesis in proper concentrations, the RNA ensembles should include both the catalytic RNA for replication and the catalytic RNAs for metabolite synthesis.

At first, these RNA ensembles could exist in the so-called Darwin ponds [48], each of them representing one communal system of replicating, and also interacting, recombining and rearranging RNAs (see [43]) in a convecting aqueous medium, something like a 'mini-Solaris' [49]. In such a pond a large mass of diverse RNA species and precursor metabolites could be accumulated and concentrated. Later, however, the communes had to be inevitably disunited into individual microsystems. The point is that, in order to have natural selection processes for further evolution, individualized micro-ensembles of RNAs, in which ribozymes–replicases, ribozymes–synthases and some ligand-binding RNAs are retained together, have to multiply, inherit useful characteristics and outgrow each other [46]. To solve the problem, usually the enfolding of self-replicating micro-ensembles in a boundary membrane is suggested (see, e.g. [46]). This way, however, seems to be unlikely in the RNA world.

An alternative way to solve the above problem can be proposed. About a decade ago the capability of RNA molecules to multiply and form *molecular colonies* on gels or other solid media containing an RNA replicase and ribonucleoside triphosphates was experimentally demonstrated [50,51] (Fig. 1). Mixed colonies of RNAs on moist clays or other solid surfaces could be the primordial evolving cell-free ensembles where some RNA molecules performed genetic functions (replication of all RNA of the ensemble) whereas other RNAs were ribozymes for catalyzing metabolic reactions or formed ligand-binding structures for selectively absorbing and accumulating substances from surroundings. The cell-free situation provided conditions for rapid evolution: the RNA colonies were not fenced off from the environment and thus could easily exchange their RNA molecules. The fact of facile dissemination of RNA molecules via the atmosphere was shown in direct experiments [50]. The rapid evolution could be facilitated also by the capability of RNA to spontaneous (non-enzymatic) *trans*-recombinations and *cis*-rearrangements [43]. In addition, the level of mutations was high due to the known inaccuracy of RNA replication mechanisms. All the above nicely corresponds to the conditions postulated by C. Woese for the ori-

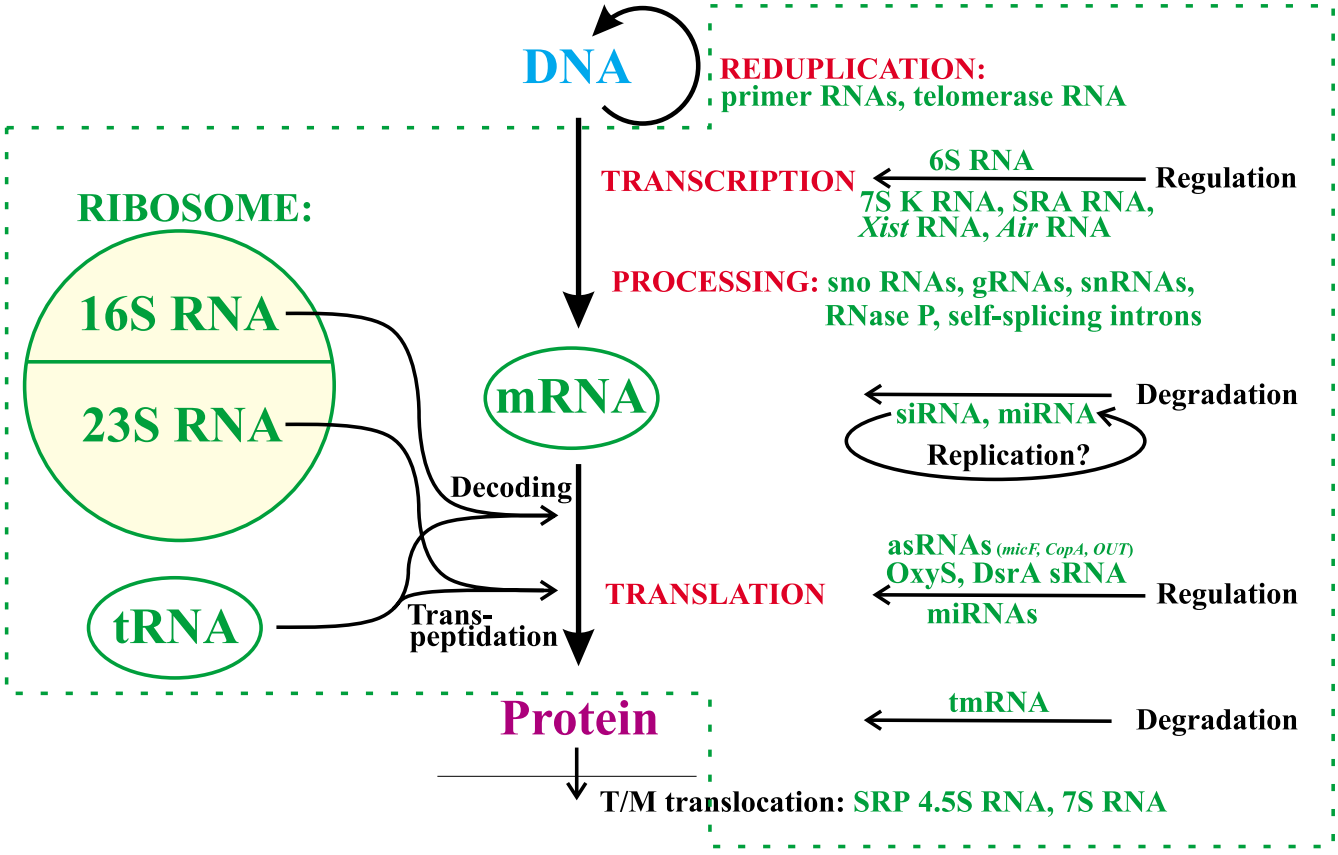

Fig. 2. Intracellular RNA world (see [53] for references).

gin of the universal precursor of living beings on the Earth: the high level of mutations, the free exchange of genetic material between cell precursors, and the communal character of the existence of these precursors when products and innovations of one quickly became the property of others [52]. In the version presented here, however, the role of the universal precursor is attributed to the cell-free form of the existence of the RNA world when neither DNA nor protein biosynthesis mechanisms had appeared yet, rather than to developed and membrane-enfolded cell precursors of the progenote type. The universal precursor could be just the world of the colonies of loosely compartmentalized RNA ensembles growing and multiplying on solid media, with lateral pandemic transfer of information. The Darwin ponds could coexist with the RNA colony world and serve as pools for massive multiplication and accumulation of the 'best' RNAs with their further dissemination. Then cycles resembling the artificial selection/evolution technique could be in operation, such as: multiplication and accumulation of a variety of RNAs in a pond (RNA pool) → their sowing and growth in the form of mixed colonies on solid surfaces → natural selection of the 'best' RNA colonies → multiplication and accumulation of the 'best' RNAs in a pond, and so on.

6. Conclusion: The contemporary RNA world

The diverse community of loose-knit and poorly individualized entities, such as mixed RNA colonies, evolved and refined into cells with their discrete individualities and their own genealogical histories (see [52]). What happened to the communal RNA world after the appearance of the protein synthesis mechanism, DNA and the membrane-enclosed cells? The community disappeared, but the RNA world in another form has been preserved, partly as developed pieces, partly as relics, in each cell of each living organism. Indeed, the cell is filled with various RNAs. The core of present-day life is the inherited and regulated pattern of protein synthesis. The ensemble of interacting RNAs of different types is known to organize the apparatus for protein biosynthesis. They include, first of all, the ribosomal RNAs forming ribosomes as protein-synthesizing machines, mRNAs providing the flow of information (programs) from DNA to the machines, and tRNAs supplying the machines with activated amino acids as material for making proteins. These three types of RNA comprise the major mass of cellular RNA and a significant fraction of the total cellular mass. Functionally, they form the soul and the staff of the process of protein biosynthesis (Fig. 2) and thus the central body of the living matter.

The intracellular world of RNA, however, is not limited to the three main RNA species. By now, the existence of a great number of minor species of cellular RNAs has been shown. The diverse minor RNAs were found to be involved in the processes of DNA reduplication and cell division, gene copying and mRNA formation, translational regulation and co-translational transport of proteins across membranes, regulation of embryogenesis and cell differentiation, determination of life span, etc. [53] (see Fig. 2). Each year, more and more new species of minor non-coding RNAs and their roles in the cell are being discovered. Until recently the role of RNA in life seems to have been underestimated, and a serious reeval-

uation of the relative contribution of the intracellular RNA world to the functioning of contemporary living beings is required.

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