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The Ribosome and the Spliceosome Are the True Ribozymes

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Recent advances in ribosome crystallography revealed an atomic resolution structure of the peptidyl-transferase active site. Similarly big progress in biochemical studies of spliceosomes provided a good basis to modify our view concerning functions of these particles. In this review the problem if the ribosomes and the spliceosomes are the ribozymes is discussed.

Key words: crystallography, 50S and 30S ribosomal subunits, ribosomal function, spliceosome, ribozyme

Introduction

Catalytic RNAs – called ribozymes – have been discovered several years ago. In 1982 Cech found that an intron of ribosomal 23S RNA precursor from *Tetrahymena thermophila* is capable to excise itself *in vitro* without any protein and external energy source [1]. At the same time Altman showed that the RNA component of RNase P (M1 RNA) of *Escherichia coli* is able to process tRNA precursors without the presence of any protein [2]. Group I and II introns as well as RNase P belong to structurally complex ribozymes, several hundred nucleotides in length [1–3]. Other classes of naturally occurring ribozymes catalyze cleavage or ligation of the RNA by transesterification or hydrolysis of phosphdiester bond. The group of small catalytic RNAs (50 to 150 nucleotides) include the hammerhead, the hairpin, hepatitis delta virus (HDV) and *Neurospora* Varkud satellite ribozymes (VS) [4,5]. Except of natural ribozymes other were synthesized and selected by SELEX approach [6]. The finding of the RNA catalytic activity was very surprising at the beginning, especially in the case of ribosome and spliceosome, which turned out to be ribozymes.

Spliceosome is a ribozyme

Most messenger RNAs encoded by nuclear genes are synthesized as pre-mRNAs, which consist of coding sequences (exons) interrupted by intervening sequences (introns). In functional mRNAs, introns are excised but exons are precisely joined to-

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gether. The process of intron removing from pre-messenger RNA (splicing) is catalyzed by a spliceosome, a large complex consisting of at least 50 proteins and five small nuclear RNAs [7]. Critical steps in that catalysis are recognition, positioning of the substrate and stabilization of the transition state of the reaction. Splicing of the group II of introns is accomplished in two steps. In the first reaction, 2′-OH group of the adenosine attacks the 5′ splice site phosphate, leaving the cleaved 5′-exon and 3′-exon intermediates. In the second step the liberated 3′-OH group of the 5′-exon attacks the 3′ splice site phosphate, releasing an intron and ligated exons (Fig. 1). One of the major questions concerning spliceosome function is the nature of the catalytic core of the machinery. Are the reactions catalyzed by RNA or by protein, or perhaps both? There are biochemical and mutational evidences implicating snRNA in *e.g.* highly conserved regions of U2 and U6 in conventional spliceosomes – in the trans-esterification reactions, but no definitive proof that they contribute to the active site. In fact, it is not clear whether the spliceosome has one active site (which would, perhaps, be remodelled between the first and second reaction) or two sites, one for each of the chemical steps. In that complex, a critical network of interactions was identified between mRNA and snRNA [8–11].

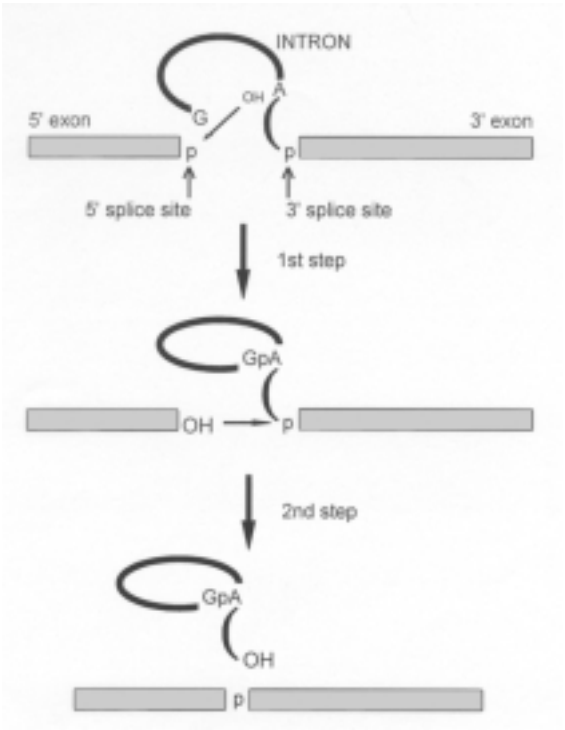

Figure 1. The two steps chemical pathway of nuclear precursor messenger RNA splicing: first 2′ hydroxyl group of adenosine (the branch point) attacks the phosphodiester bond at the 5′ splice site generating the cleaved 5′ exon and lariat intron3′/exon intermediate; second step the liberated 3′-OH of the 5′ exon attacks the 3′ splice site phosphate, releasing the intron lariat and ligated exon.

For spliceosome assembly and intron recognition five snRNAs are required: U1, U2, U4/U6 and U5. U1 and U4 are released from the spliceosome complex before catalysis occurs, so only U2, U5 and U6 potentially participate in reaction of splicing. All nucleotides involved in interaction are highly conservative. There are experimental evidences that U6 and U2 snRNA interact with the intron near the 5′ splice site and branchpoint *via* helices [10]. The adjoining U2/U6 helices I, II and III could juxtapose these two reactive sites for the first phosphoryl – transfer step. The highly conserved loop of U5 snRNA had been implicated in positioning the exons for ligation during the second step of the splicing [10]. Additional interactions were found between the first and last guanosine residue of the intron and an invariant U6 RNA residue near the 5′ splice site helix with bulged U2 RNA residue in helix I. U6 is the best candidate for direct role in catalysis. It acts not only near or at the active site of spliceosome but it is very sensitive to mutations and modifications [12]. Essential cofactors in catalysis, especially for RNA enzymes, are very often metal ions [13,14]. It has been demonstrated recently that the spliceosome binds two Mg^{2+} ions and no other divalent cations are required [15,16]. The search for catalytic metal ion binding sites in the spliceosome has pointed to a conserved structure in U6 snRNA. Yean *et al*. have identified that Mg^{2+} is coordinated by U80 of U6 what stabilizes the leaving group at the 5′ splice junction [17].

Up to now the role for the proteins in the splicing is not well documented. Several proteins have been crosslinked to mRNA nearby the catalytic site, but only Prp8 (highly conserved protein 62% identity from yeast to humans, a component of the spliceosome catalytic core) forms extensive bonds within U5 and U6 snRNAs [18,19]. Up till now there are few evidences that protein Prp8 plays a crucial role in the chemistry of splicing. It stabilizes tertiary RNA interaction and facilitates formation of the catalytic core by promoting the folding of the active RNA structure. Thus, there is a possibility that Prp8 acts as a cofactor to RNA enzyme. All experiments concerning the spliceosome provided evidences that it is an RNA enzyme [16,20].

Three-dimensional structure of 50S ribosomal subunit

The ribosome is a site for translation of genetic information encoded in messenger RNA (mRNA) into protein. This process requires several factors for its initiation, elongation and termination. The prokaryotic ribosome is a large complex build up of two subunits 30S (0.85 MDa) and 50S (about 1.75 MDa). The bacterial large ribosomal subunit consists of 23S rRNA, 5S rRNA and 31 proteins (called L1 – L31). Recently, crystal structure of 50S ribosomal subunit from *Haloarcula morismortui* was determined with 2.4 Å resolution [21–26]. The crystallographic map clearly shows the density for 2711 of the 2923 nucleotides of 23S rRNA, all 122 nucleotides of 5S rRNA and 27 out of 31 of ribosomal proteins [21]. Secondary structure of 23S rRNA can be divided into six domains (I–VI) connected with a central loop (Fig. 2a). Analysis of crystal of 50S subunit for the first time exhibits a whole picture of 5S rRNA molecule. It does not interact extensively with 23S rRNA, although a few bonds were

observed. These two molecules are connected together mainly through protein interactions. 5S rRNA molecule consists of three stems radiating out of loop A. In contrast to previous models, where domains β and γ of 5S rRNA interact with each other, there is no direct contact between them in ribosome (Fig. 2b). Domains β and γ of 5S rRNA are hugging the top of ribosome subunit covering it from the site of L7/L12 proteins.

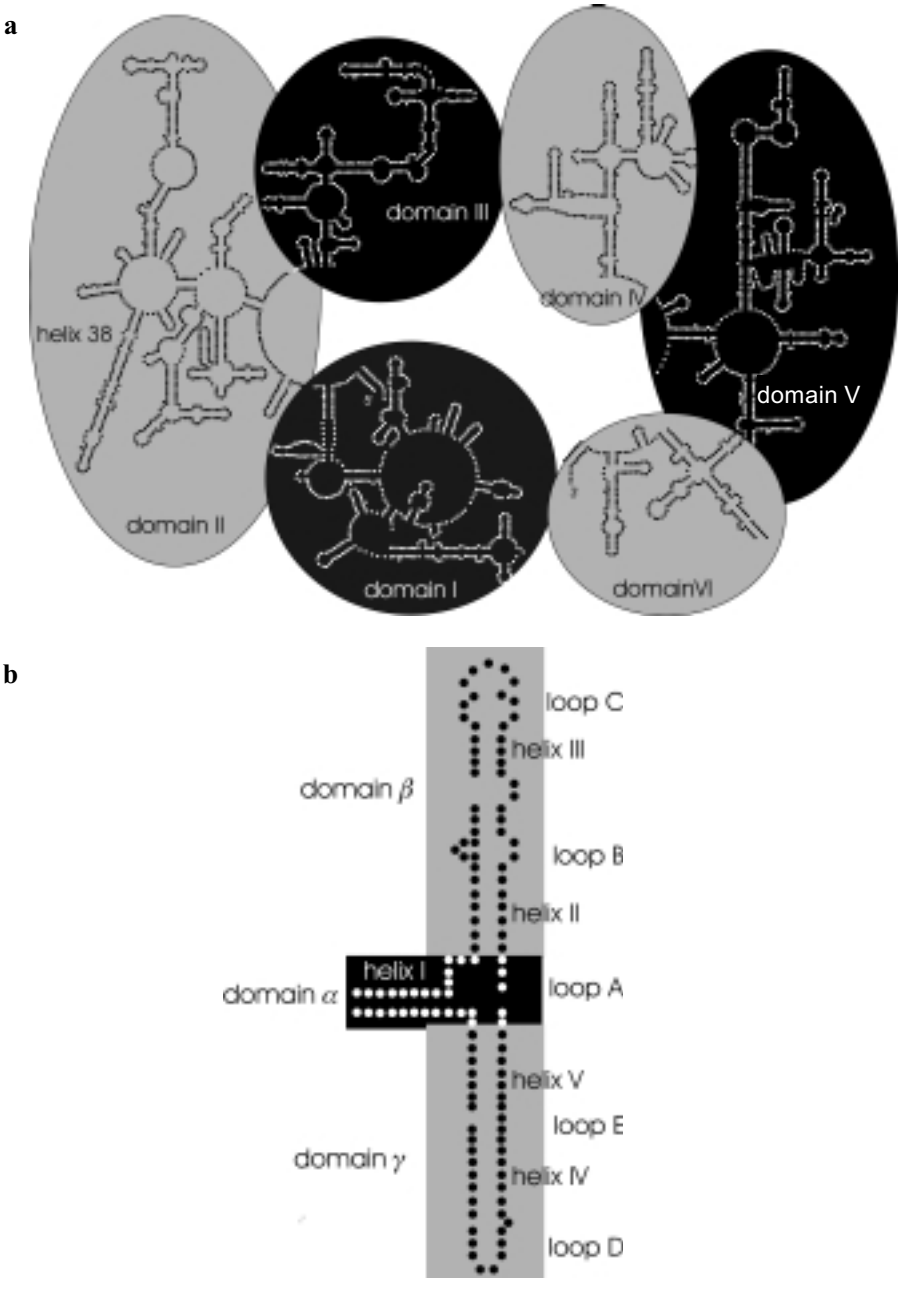

Figure 2. Secondary structure of a) 23S rRNA, b) 5S rRNA, and c) 16S rRNA (continuation on next page).

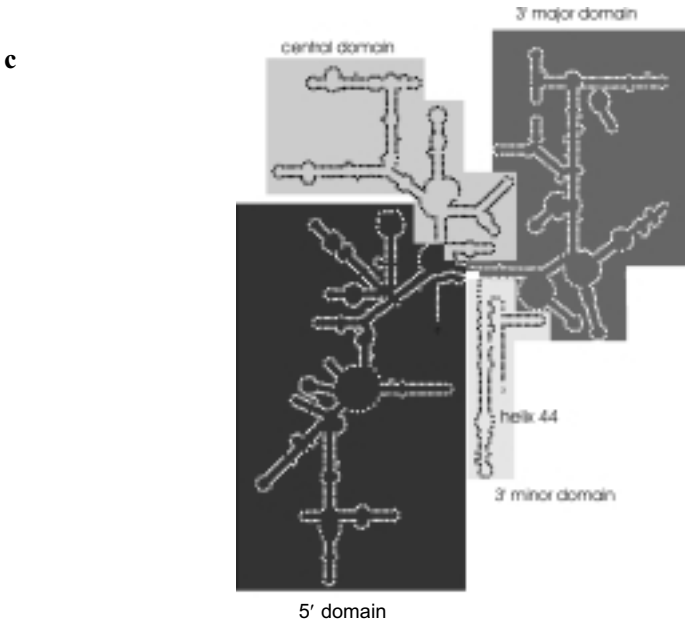

Figure 2. Secondary structure of a) 23S rRNA, b) 5S rRNA, and c) 16S rRNA.

These differences between structure of 5S rRNA, observed in 50S subunit and proposed for free molecule in solution, suggest a rather big flexibility of the particle [27]. Proteins of the large subunit, with the exceptions of L1, L10, L11 and L12 (Fig. 3), are dispersed throughout the structure and concentrated on its surface, but they are absent from the regions significant for protein synthesis (the 30S subunit interface and the peptidyl transferase active site) and they do not extend significantly beyond the RNA core.

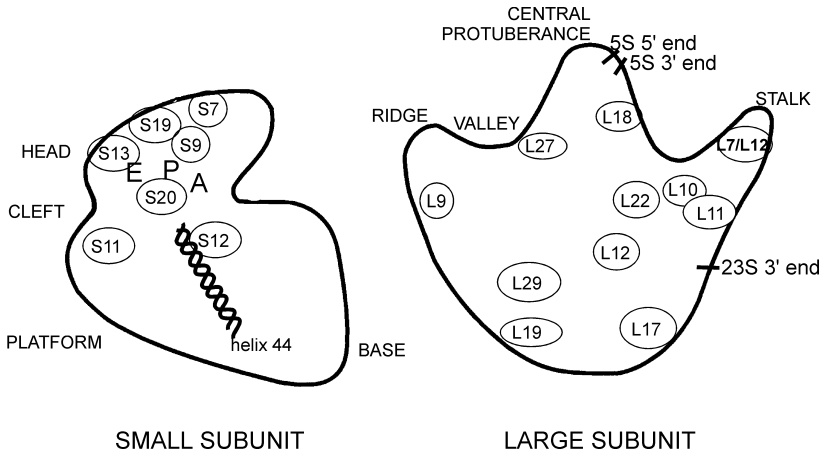

Figure 3. Summary map of proteins and functional sites on 30S and 50S ribosome subunits.

Structure of 23S RNA from crystal of 50S subunit shows that 1157 nucleotides out of 2923 make at least van der Waals contact with protein [21,24,25]. All proteins, except of L12, interact directly with RNA. Protein L22 interacts with all six domains of 23S rRNA. Seven proteins interact only with one domain of 23S among them L1, L10 and L11 are directly involved in the protein synthesis. Another three L24, L29 and L18 stabilize the tertiary structure of 23S rRNA. L7 is the most puzzling protein from all other, it interacts with single sequence in domain I, and it is close to L1, which appears to be important for E-site function, so could be that L7 is involved in this activity [28].

Three-dimensional structure of 30S subunit

A small bacterial ribosome subunit (30S) consists of 16S rRNA and 21 proteins (named S1 - S21). This particle is divided into four domains (5′-, central-, 3′-major and 3′-minor domains) of 16S rRNA as observed in the secondary structure (Fig. 2c). The 3′-minor domain forms an extended helix 44, which runs down the long axis of 30S subunit surface interacting with the 50S. A major function of the 30S subunit is decoding of mRNA, where tRNA anticodons pair with mRNA within the A-site. Crystal structure of 30S rRNA from *T. thermophilus* was solved and refined with 3 Å resolution [24]. The secondary structure of 16S RNA (1518 nucleotides) contains over 50 regular helices connected by irregular single stranded loops. In crystal structure these loops look like double stranded extensions of neighbouring regular helices. The proteins are concentrated in the top, sides and back of 30S subunit. None of the proteins binds entirely inside an RNA, although S20 binds between 3′ minor and 5′ domains. Similarly to the 50S subunit, the 30S interface is largely free of protein with exception of S12, which lies near the decoding site at the top of the long helix 44. Functionally the most important parts of the 30S subunit are binding sites for substrates: A (aminoacylo-tRNA), P (peptidylo-tRNA) and E-site (exit site for free tRNA). The P-site is occupied by the tip of the steam-loop (H6), which mimics the tRNA anticodon. It contacts of 16S RNA (nucleotides: 1338–1341 and 1229–1230) and fragment of the carboxy-terminal tails of proteins S13 and S9. The A-site is more wider and shallower than P- or E-sites. Such geometry may reflect the need to allow rotation of the codon-anticodon helix during or after GTP hydrolysis by EF-Tu. The only protein, which appears to participate directly in decoding of mRNA, is S12 and 12 residues of C-terminal parts of S19. Unlike to aminoacyl-tRNA binding site and peptidyl-tRNA binding site, the E-site consists mostly of protein S7 and S11. The position of highly conserved β–hairpin of S7 indicates that it might help dissociate the E-site codon-anticodon.

The mechanism of the peptide bond formation

Aminoacyl-tRNAs and peptidyl-tRNAs are the substrates for the reaction catalyzed by the large ribosomal subunit. The first one binds to A-site and the second to the P-site. The crystal structure of 50S subunit suggests that A2486 is involved in pep-

tide bond formation. N3 of that conserved adenosine, placed in catalytic site, abstracts a proton from the α-amino group of A-site bound aminoacylo-tRNA. It facilitates the nucleophilic attack of this nitrogen on the carbonyl carbon, acylating the 3′OH group of the tRNA in the P-site (Fig. 4) [22]. This mechanism is supported by the biochemical data obtained by Yarus *et al*. [29]. They used puromycin (CCdA-p-Puro) (Fig. 5) as an aminoacylo-tRNA analog which interacts with A-site and mimics the tetrahedral carbon intermediate. The crystal structure of 50S subunit in complex with CCdA-p-Puro and with mini helix analog of aa-tRNA shows that CCdA binds in P-site [30], while CCA is visible in A-site [31]. The puromycin group in both cases is located in the same site and there is no protein around that place, so the catalytic activity must depend entirely on RNA. Both puromycin moiety and A-site analog of aa-tRNA bind to the 50S at the bottom of the large and deep cleft of polypeptide exit tunnel [30,31]. This site is surrounded by nucleotides belonging to the central loop of 23S rRNA domain V, *e.g*. the peptidyl transferase loop. The RNA that surrounds the substrate is closely packed, what resembles the active site of protein en-

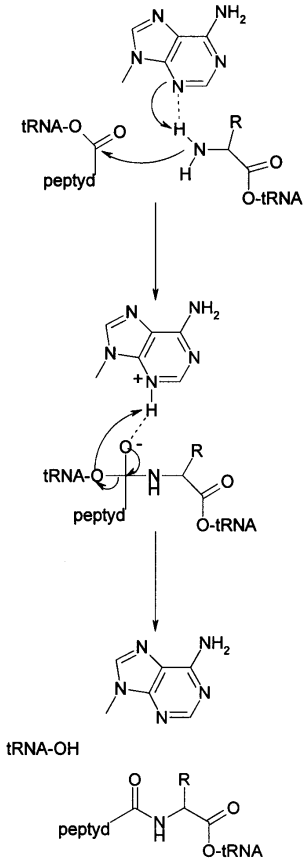

Figure 4. The proposed mechanism of peptide synthesis catalyzed by ribosome discussed in the text.

zyme and the nucleotides, which are in contact with the inhibitor are more than 95% conserved in all kingdoms. The critical N3 of A2486 is located about 3 Å from the carbonyl oxygen of a nascent peptide bond and the amide corresponding to the nitrogen of the peptide bond being synthesized (Fig. 6). The most important issue here is pK_a of discussed N3. In the crystal structure it is protonated at pH 5.8, but at physiological pH a significant fraction is in the unprotonated form functions as a general base. The following processes are possible: the N6 of A2486 interacts with the O6 of G2482 and G2102, the N2 of G2482 also interacts with oxygen from phosphate group of A2485. The phosphate of A2485 interacts electrostatically changing the pK_a of A2486 or alternatively it abstracts the proton from N2 of 2482 and neutralizes its negative charge (Fig. 6). The interaction of amino tautomer of G2482 with A2486 can stabilize the imino group of A2486, what result in a negative charge of N3. The A2486 and G2102 are completely conserved in ribosomes of all organisms except of three archea and the same eubacteria [22].

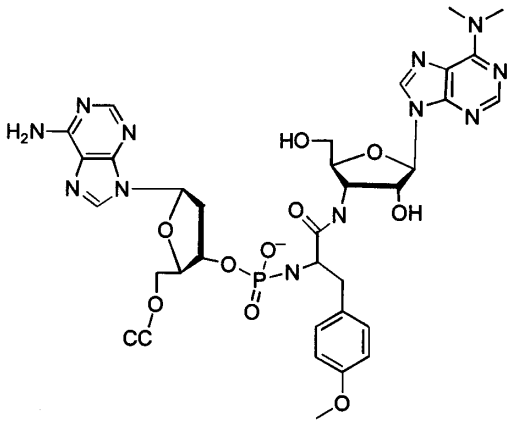

Figure 5. The transition state analog formed by coupling the 3′OH of CCdA to the amino group of the O-methyl tyrosine residue of puromycin *via* a phosphate group, CCdA-Puromycin (Yarus component).

A similar mechanism for peptide bond formation has been proposed for peptide synthesis at high pressure. Phenylalanyl- $tRNA^{Phe}$ and dipeptide Gly-Gly, exposed to 6 kbar for 18 hours at room temperature, formed tripeptide Gly-Gly-Phe. It seems that carbonyl group is activated at high pressure, what stimulates the nucleophilic attack of nitrogen of the amino group of amino acid bound to tRNA. One can suggest that RNA could catalyze different reactions what means that RNA is an enzyme [32].

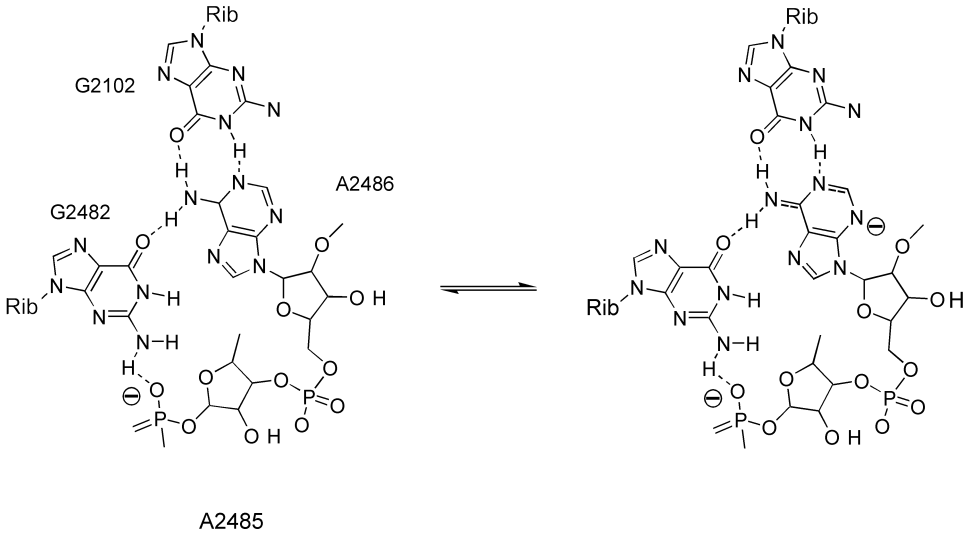

Figure 6. The normal and imino tautomeric forms of G2482 and A2486 that could be stabilized by the buried phosphate of residue 2485.

Conclusions

The high resolution structure of the large ribosomal subunit shows that the ribosome is a ribozyme [3]. Similar conclusion concerns the spliceosome, although three dimensional structure of splicing machinery is not known. It means that the processes carried out by ribosome and spliceosome are catalyzed by RNA. In both cases proteins act as cofactors, stabilizing and orienting the floppy RNA into a specific active structure. It means that ribosome and spliceosome are the ribozymes. Of course the question arises why does nature use RNA to catalyze the protein synthesis? One of the possible answers is that in primordial world RNA provided both genetic information and catalytic function thus, earliest protein synthesis had to be catalyzed by RNA.

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