neurodegenerative diseases when it was found to be a minor component of amyloid plaques in Alzheimer's disease [19].

Alternatively, it is possible that there is an inhibitor of prion generation and propagation whose activity is inhibited by an excess of another prion. Excess Hsp104p cures [PSI$^+$] [20], excess Ydj1p (an Hsp40) can cure [URE3] [21], and Sis1p (another Hsp40) cures [RNQ$^+$] [22]. Overexpression of Ssb members of the Hsp70 family promote curing by Hsp104 [23]. Amyloid or aggregates of one overproduced protein could tie-up one or more of these factors, preventing their anti-prion action and accounting for the Pin$^+$ phenotype. Distinguishing these two models will not be easy, but it seems likely that continued study of the yeast models of amyloidosis and prion diseases will open new ideas and approaches to these difficult human diseases.

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Creative catalysis: pieces of the RNA world jigsaw

Jeremy M. Murray and Jennifer A. Doudna

Novel ribozymes produced by *in vitro* selection techniques provide insights into the possible mechanisms of protein synthesis evolution. The availability of such ribozymes also paves the way for experiments to explore the evolution of RNA–protein enzymes.

One of the biggest paradigm shifts in the field of biochemistry in the 20th century resulted from the discovery of RNA enzymes (ribozymes). The series of classic experiments involving *Tetrahymena* pre-rRNA helped to confirm the importance of ribozymes in cellular RNA processing [1–3] and lent increased credibility to the RNA world hypothesis [4]. In this envisioned world, RNA served both as the genetic material and the principal cellular enzyme, and was probably assisted in the latter role by metals, pyridines, amino acids and other small-molecule cofactors. The RNA world hypothesis posits that as cellular metabolism became more sophisticated, increasing demands on biocatalysts provided the impetus for the transition to protein enzymes. Descendants from this proposed RNA-dominated era inhabit the world today in the form of naturally occurring ribozymes present in organisms ranging from bacteria to humans. Until recently, none of these ribozymes has provided insight into the chicken and egg paradox surrounding the evolution of protein synthesis. However, evidence obtained through the application of *in vitro* selection techniques now suggests that RNA could have been the victim of its own initial success.

According to RNA-world theorists, protein enzymes evolved from ribozymes through the process of natural selection. But how might this transition have occurred? Logically, a first step must have been the emergence of ribozymes that catalyzed the reactions necessary for protein synthesis: amino acid activation, aminoacyl-tRNA synthesis and peptide-bond formation. However, no naturally occurring ribozymes that catalyze the first two reactions are known. Furthermore, although the ribosome catalyzes peptide-bond formation using an

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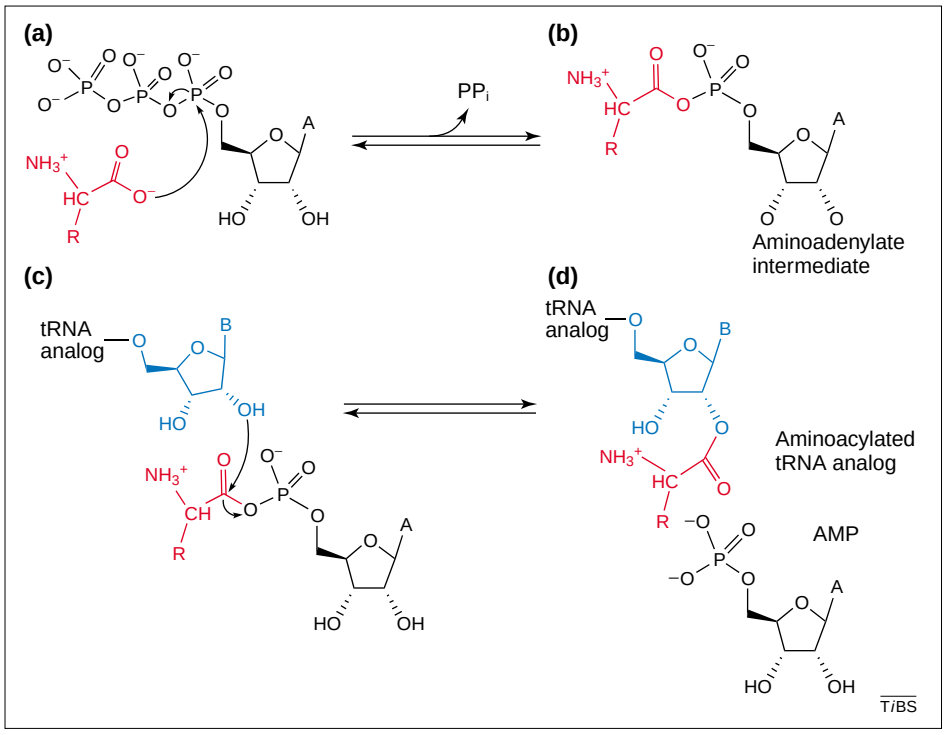

Fig. 1. Mechanism of aminoacylation of tRNA by either aminoacyl-tRNA synthetases or ribozymes. (a→b) The amino acid (red) is activated via an aminoadenylate intermediate in which a highly reactive mixed anhydride forms between the amino acid carboxylate and the α-phosphate of ATP. (c→d) The amino acid is transferred from the aminoadenylate intermediate to a tRNA or tRNA analog (blue) by formation of an ester linkage between the amino acid and the 2′(3′) terminus of tRNA.

active site composed of RNA, at present, its large size and complexity preclude an understanding of its evolutionary origins.

Given these observations, a natural question is whether relatively small RNA molecules, such as might have arisen in an RNA world, have the potential to catalyze chemistry required for peptide synthesis. Whereas protein enzymes have evolved to catalyze a wide range of reactions, the eight naturally occurring ribozymes are limited to phosphodiester-transfer chemistry. Although this might imply an inherent limitation for RNA, several lines of evidence suggest otherwise. Initial studies of ribozyme reaction mechanisms revealed several properties that are common to protein enzymes. First, rate enhancements can be substantial. For example, the rate constant for chemistry of the self-cleaving hepatitis delta virus ribozyme is estimated at 10^2 to 10^4 s^{-1} [5], which is close to the maximal cleavage rate of ribonuclease A (1.4×10^3 s^{-1} at 25°C) [6]. The *Tetrahymena* group I self-splicing ribozyme also has a catalytic rate constant that is comparable to rates of protein enzymes [7]. Second, ribozymes can use co-factors during catalysis. Group I introns require an exogenous guanosine nucleotide for activity, positioning the 3′-hydroxyl group of a bound guanosine nucleotide for nucleophilic attack of the phosphate at the 5′-splice site. Third, although many ribozymes catalyze reactions *in cis*, ribonuclease P is an example of a true biological catalyst in the sense that it processes substrate RNA molecules *in trans*. All these properties led to the conclusion that ribozymes share fundamental features of protein enzymes that enable them to function as efficient biocatalysts *in vivo*.

More recent structural and mechanistic investigations have led to the idea that ribozymes are surprisingly similar to protein enzymes when viewed at a detailed chemical level. Molecular structures have revealed that both types of catalysts fold into specific three-dimensional shapes that can harbor deep grooves and solvent-inaccessible active sites. These tertiary structures facilitate catalysis, in part, by orienting substrates adjacent to catalytic groups and metal ions. Although the inherent chemical differences between protein enzymes and ribozymes might have implied distinct catalytic strategies, mounting evidence suggests notable mechanistic parity for these catalysts. The *Tetrahymena* group I self-splicing intron is a metalloenzyme that catalyzes an S_N2 phosphodiester transfer via a magnesium-dependent two-step mechanism similar to that of DNA and RNA polymerases. The first step involves the in-line nucleophilic attack of the 3′-OH of the guanosine cofactor on the scissile phosphate at the 5′-splice site, cleaving the RNA backbone in a concerted reaction. In the second step, the newly formed 3′-OH of the 5′-exon nucleophilically attacks the 3′-splice-site junction. Similarly, the hammerhead ribozyme uses an S_N2 mechanism involving a metal ion cofactor [8]. However, other ribozymes might not be metalloenzymes. Crystal structures of the hepatitis delta virus and hairpin ribozymes both suggested that active-site nucleotides can directly facilitate chemistry via a general acid–base mechanism [9,10]. The crystal structure of the ribosome, together with biochemical evidence, indicated that this ribozyme might also use acid–base chemistry. Efficient proton donation or acceptance by a ribozyme requires the perturbation of one or more nucleotide pK_a values to near-neutral, a phenomenon that has been observed in several different RNAs [11–15].

Although the types of reactions catalyzed by naturally occurring ribozymes appear limited, ribozymes obtained through the application of *in vitro* selection techniques [16] reveal that they can achieve a level of biochemical sophistication necessary to support cellular metabolism. Starting with a pool of random RNA sequences, molecules possessing a desired activity are isolated through successive cycles of activity selection, reverse transcription of the 'winners' into DNA, and amplification of those sequences by the polymerase chain reaction (PCR). Because of technical constraints on the size of the starting pool, ribozymes identified in this way are typically 50–100 nucleotides in length, comparable to the size of the hairpin or hepatitis delta virus ribozymes but less than half the size of self-splicing introns. This SELEX (systematic evolution of ligands by exponential amplification) methodology has facilitated tantalizing recent progress in the elucidation of missing links necessary to make the transition from an RNA world into

contemporary biology. Through the clever use of selection conditions, ribozymes able to carry out the two reactions catalyzed by tRNA synthetases (Eqns 1 and 2; Fig. 1) during protein synthesis have been identified.

$$NH_3^+(R)CHCOO + ATP \leftrightarrow NH_3^+(R)CHCOO\text{-}AMP + PPi \quad [1]$$

$$aa\text{-}AMP + tRNA \leftrightarrow aa\text{-}tRNA + AMP \quad [2]$$

Isolation of ribozymes able to catalyze the first reaction had proven difficult because of the unstable nature of the aminoacyl adenylate (aa-AMP) intermediate. The reaction occurs through the nucleophilic attack of the carboxylate group of the amino acid on the 5′-α-phosphate of ATP with the release of pyrophosphate. The use of low pH in the selection conditions allowed the isolation of ribozymes capable of catalyzing this reaction at a rate of 1.1 min^{-1} in 5 mM Ca^{2+}, 50 mM NaCl and 40 mM cacodylate (pH 4) [17]. The second step in translation of RNA to protein proceeds through the attack of a terminal 2′(3′) ribose hydroxyl on the carbonyl carbon of the aa-AMP intermediate, resulting in the esterification of the 2′(3′) terminus of tRNA and the release of AMP. This latter reaction can be catalyzed by calcium-requiring ribozymes with the speed and specificity of aminoacyl-tRNA synthetases [18].

These new ribozymes place some of the missing pieces in the RNA world jigsaw by demonstrating that RNA can conduct the essential reactions required to achieve template-directed protein synthesis. This is an important step towards the construction of a system in which ribozymes are capable of producing peptides. Further experiments to identify the conditions under which short peptides could be made from these *in vitro* selected ribozymes might allow the isolation of ribozymes that enhance their own catalytic efficiency by using peptide ligands and cofactors. Indeed, recent advances in the *in vitro* selection of nucleoprotein enzymes [19] provide insights into the increased ribozyme efficiency that can be obtained with assistance from protein cofactors. The generation of ribozyme-assembled peptides and their positive influence on ribozyme activities is an exciting, and increasingly realistic, possibility. Although it will not prove the existence of an RNA world, such a system would provide an extraordinary opportunity to explore the evolutionary link between ribozymes and their protein counterparts.

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