



INSTITUT PASTEUR

Research in Microbiology xx (2009) 1–6



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The evolving ribosome: from non-coded peptide bond formation to sophisticated translation machinery

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Received 2 April 2009; accepted 4 July 2009

Abstract

Structural analysis supported by biochemical, mutagenesis and computational evidence, revealed that the contemporary ribosome's active site is a universal symmetrical pocket made of ribosomal RNA. This pocket seems to be the remnant of the proto-ribosome, a dimeric RNA assembly evolved by gene duplication, capable of autonomously catalyzing peptide bond formation and non-coded amino acid polymerization. © 2009 Elsevier Masson SAS. All rights reserved.

Keywords: Ribosome; Evolution; Proto-ribosome; Peptidyl transferase center

1. Introduction

Contemporary translation is a complex process performed by the ribosome, a naturally occurring ribozyme that outlived the transition from the prebiotic era to modern life. These universal catalytic machines are riboproteins of molecular weights ranging from 2.5 MDa for eubacteria and archaea to 4 MDa for early eukaryotes and highly developed mammals. Remarkably, despite the size difference, the functional regions of ribosomes, the decoding center and the peptidyl transferase center (PTC) from all life domains are composed solely of highly conserved ribosomal RNA (rRNA).

Approaches that have been used for the identification of the predecessor of the contemporary ribosomes include comparative sequence analysis [35], synthesis of RNA constructs exposed to *in vitro* selection [4], investigation of the interactions of the peptidyl transferase center (PTC) with its neighboring proteins [51], analysis of all internal ribosomal interactions [9], dissection of the molecular anatomy of the PTC [42] and analysis of selected functional steps in the three domains of Life [33]. However, until recently [3], none of

these studies was focused on the spontaneous appearance of prebiotic machinery for peptide bond formation.

Here we discuss biochemical evidence supporting the existence of an ancestral dimeric proto-ribosome, as well as of materials that could serve as its substrates, i.e. amino acylated mono-, di- and three-nucleotides. These include the development of structural tools for approaching one of the key questions in evolution, namely: did the ancient translation apparatus survive selection pressure and are its vestiges embedded within the modern ribosome?

2. A symmetrical site for peptide bond formation

The PTC, i.e. the site of peptide bond formation, is located at the heart of the contemporary ribosome in the midst of a symmetrical 'pocket-like' structure [1–3,6] (Fig. 1), an unusual feature within the otherwise asymmetric ribosome. This region exists in all known ribosomes high resolution structures [5,23,29,48,49,59] and its inner part displays resistance to mutations [47]. It encompasses 180 nucleotides of which the backbone folds, irrespective of the nucleotide sequences, are related by pseudo two fold symmetry.

Each half of this symmetrical 'pocket-like' region hosts one of the ribosome's substrates, namely the amino acylated and the peptidyl tRNA molecules. Furthermore, the elaborate

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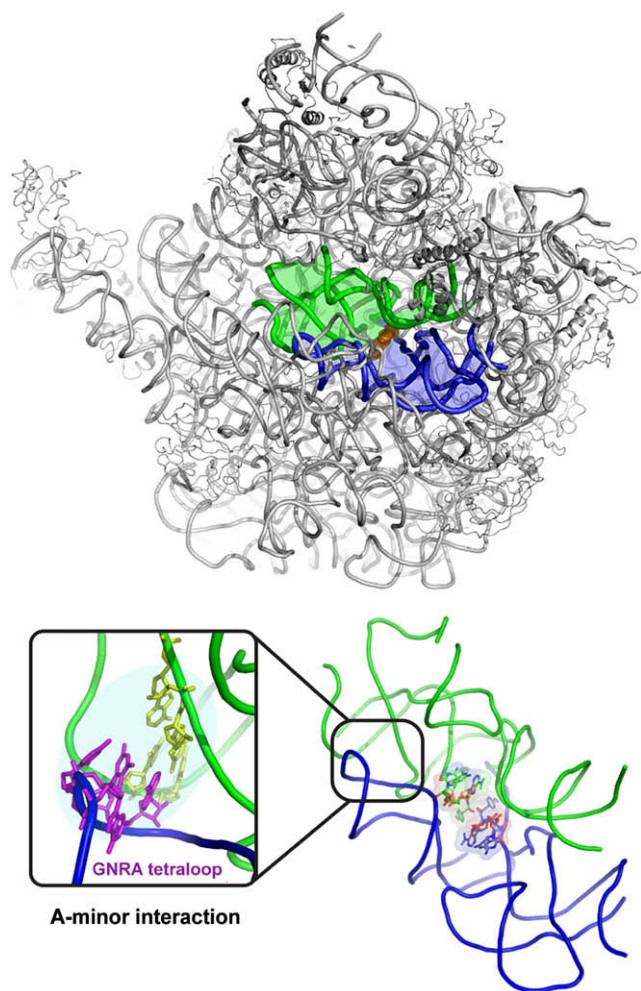


Fig. 1. Top: the symmetrical region within the large ribosomal subunit. The A-region is shown in blue, the P-region in green, and the substrate position is indicated by the orange object. Bottom: the GNRA tetramer and the A-minor interaction between the A-region (blue) and the P-region (green).

architecture of this structural element provides the framework for the ribosome catalytic contribution, as it positions the ribosome's substrates in favorable stereochemistry for peptide bond formation [1,2,6,8,22,36,58] and for substrate-mediated catalysis [57,64,65,7]. Additionally, by encircling the PTC it confines the void required for the motions involved in the translocation of the tRNA 3' end, which, in turn, is necessary for the successive peptide bond formations, enabling the amino acid polymerase activity of the ribosome [1–3,7].

All of the rRNA entities of the symmetrical region possess the common stem-elbow-stem (SES) structural motif, and are believed to be capable of self-assembly and dimerization [2,3]. Based on the above observations we have proposed that the ancient machinery that could form peptide bonds was made exclusively from RNA molecules, utilizing substituents available in the primordial soup, such as short RNA chains that could acquire conformations that are sufficiently stable to survive changing evolution stresses. The assumption of a self assembled ribosomal active site, which is still implanted in the modern ribosome, triggered experiments aimed at revealing

self folding of RNA chains, as well as their tendency to dimerize and to acquire the structural elements necessary for amino acid polymerization (see below).

3. Origin of proto-ribosome entities in the prebiotic era

The existence of RNA oligomers in the prebiotic era is widely accepted [26,27,39,54]. Support for this assumption is the non-enzymatic synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions [43,53], and the demonstration that RNA oligomers can be obtained non-enzymatically from such activated RNA precursors [40]. Among the pool of potential RNA structural elements existing in the RNA world, it is widely assumed that the SES motif was a common structural element, as it is the main building block of RNA functional molecules, such as gene regulators, riboswitches, RNA polymerases, ribozymes catalyzing the phosphodiester cleavage, RNA processing and RNA modification [13], believed to be relics from the prebiotic world.

It is likely that owing to self complementarity, RNA chains composed of SES motifs could self fold and that these self-folded RNA chains formed stable molecules [45]. Consequently, RNA chains of SES motifs and lengths sufficient for the construction of basic elements of the modern ribosome active site, could have existed in the prebiotic period [15,17]. Importantly, the SES motif appears also in many contemporary RNA molecules, some of which are believed to co-evolve with the translation machinery, such as tRNA [11,12,15,34,37,46,56,62]. Moreover, the possible localization of the proto-ribosome or its remains within the symmetrical region of the contemporary ribosome hints that the proto-ribosome was formed by dimerization of self folded SES chains, as observed for similar systems [10,28,29,44,52].

Dimerization in a symmetrical manner of self folded SES motifs of identical, similar or different sequences could have occurred spontaneously and could have resulted in a 'pocket-like' structure formation. Stabilization of such dimers could be achieved by various tertiary interactions, among them the common GNRA (N stands for any base, R stands for a purine, G and A are guanine and Adenine) scheme [41], which includes the abundant and ubiquitous structural motif called 'A-minor' (Fig. 1) [9,10,26,38] that stabilizes RNA tertiary and quaternary structures, similar to the interaction within the 'pocket-like' entity in the contemporary ribosome. Based on the presumed capability of the prebiotic 'pocket-like' entity to host substrates such as spontaneously produced amino-acids conjugated with single or short oligonucleotides [21,25,30,31] and to catalyze peptide bond formation [1–3,6], it is suggested that the symmetrical region of the modern ribosome originated from a symmetrical proto-ribosome.

Among these 'pocket-like' molecular dimers, the most stable constructs might have survived under various environmental conditions. Those entities that could accommodate suitable substrates at stereochemistry enabling peptide bond formation might have been evolutionarily favored, since in addition to the inherent ability of RNA to replicate, these specifically functioned as proto-ribosomes in the prebiotic era

and later became the ancestor(s) of the active site of the contemporary ribosome. It is likely that some of these entities originated by fusion of two different or identical sequences, resembling a gene elongation event: a type of gene duplication-fusion particularly important for the accretion of gene size [18,19]. Among the entities that survived the evolution pressures, those possessing catalytic abilities or capable of performing other supporting functions, e.g. stabilizing the proto-ribosome entity, were selected.

The feasibility of existence of a dimeric proto-ribosome capable of peptide bond formation is being assessed by testing various RNA chains that may acquire the SES general motif (Fig. 2) for their tendency to dimerize and for their ability to form peptide bonds. In particular, experiments aimed at determining the dimerization tendency of various RNA chains designed to possess the main structural motif of the suggested proto-ribosome, namely the SES motif, were conducted in our laboratory on several RNA sequences. Data obtained indicate 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 (Fig. 3). Polyacrylamide gel electrophoresis, performed on various constructs under non-denaturing conditions showed a relationship between the tendency to dimerize and the sequence of the RNA chain, in accordance with previous data [24]. Slowly migrating bands, indicative for the presence of dimers, were detected for pairs that had been selected to mimic natural sequences as well as for RNA entities expected to possess the SES motif constructed with non-natural sequences.

Indications for a compact ‘pocket-like’ structure formation, rather than an extended structure formed *via* loop–loop interactions, were obtained by a series of deletions/insertions of base-pairs, resulting in slightly shortened or extended helices. Thus, whereas in the case of dimerization by loop–loop interactions, which should lead to extended structures, the lengths of the stems should hardly affect the level of dimerization, the structural nature of the pocket-like entity is expected to be dependent on the lengths of the stems composing it. Further functional experiments, exploring the

peptidyl transferase (PT) activity as well as the stabilization properties of a large variety of the RNA dimers are being carried out along with their structural analysis. The potential involvement of A-minor interactions in the stabilization of the proto-ribosome dimer was demonstrated by obtaining a significantly higher dimer concentration when a GNRA tetra loop was incorporated instead of the CUUCGG loop, which connects RNA stems that protrude out of the region and that have been suggested to represent the proto-ribosome within the contemporary ribosome. Further modifications, such as mutations in which the third nucleotide of the GNRA motif was replaced, did not yield dimers, thus implying the existence of functional selection at the molecular level (Davidovich et al., to be published).

4. Discussion

In the early terrestrial environment, the genetic information that was embedded in the RNA sequences could lead to self replication and to phenotypes with catalytic properties [14,15,16,31,32,40,50,54,60,61,63]. In the case of the proto-ribosome, it is likely that the more efficient and more stable RNA dimers that functioned as proto-ribosomes by positioning the substrates in a spatial arrangement similar to the modern one, could have autoreplicated. Thus, the surviving ancient pockets became the templates for the ancient ribosomes. In a later stage these molecular entities underwent optimization from non-genetic peptide bond formation towards performing genetically driven translation.

The transition from a molecule forming peptide bonds to an elaborate apparatus capable of decoding genetic information seems to be coupled with the evolving genetic code and the proto-ribosome substrates. A higher level of efficiency could have been achieved by the increase of the proto-ribosome size by the creation of a supporting environment alongside the elongation of the substrates from the minimal mono- or oligonucleotide/amino acid conjugates, towards the modern tRNA. The conversion into longer compounds with a contour that can complement the inner surface of the reaction pocket

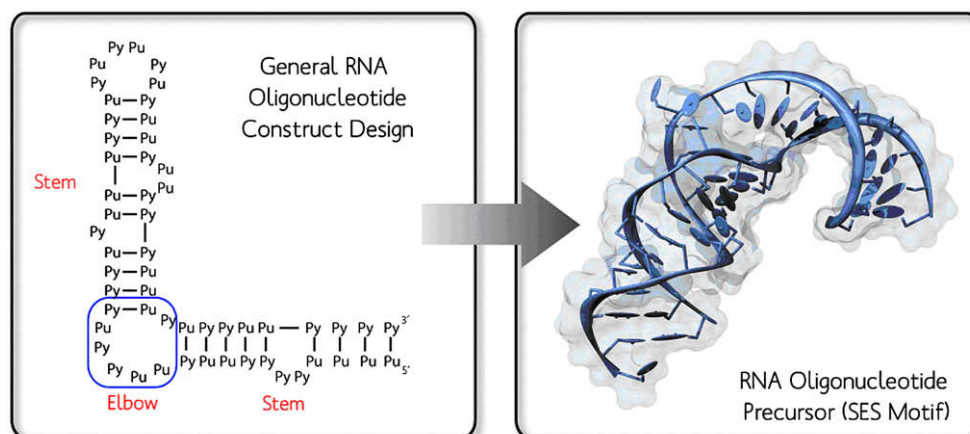


Fig. 2. Left: general design of the RNA oligomers that adopt the stem-elbow-stem (SES) motif. Right: 3-Dimensional representation of the SES motif.

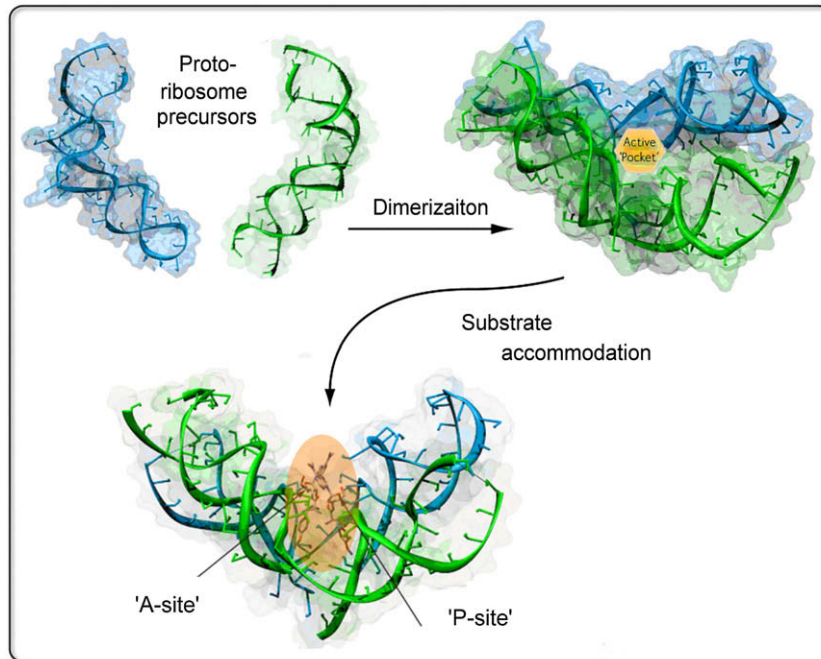


Fig. 3. Schematic representation of 'pocket-like' proto-ribosome formation from an SES RNA precursor, showing simple catalytic peptidyl transferase activity.

occurred concurrently with proto-ribosome mutational optimization, aimed at accurate substrate positioning, which in the modern ribosome is governed by remote interactions between the RNA and the cavity leading to the PTC [6,64]. This pathway is in accordance with the fact that in the contemporary ribosome the symmetry is related to the backbone fold and not to the nucleotide sequence, thus emphasizing the superiority of functional requirement over sequence conservation.

A substantial increase in the catalytic rate could be obtained by the inclusion of peripheral elements, as observed for other ribozymes [55]. These additionally recruited structural elements could have been RNA chains or elongated

oligopeptides that interacted with the proto-ribosome and its surroundings in a manner resembling the protein-RNA interactions in the modern ribosome. These could contribute to the suitability of the apparatus to act as an efficient machine and to support the emergence of genetic code translation. Mutational optimization that facilitated distinction between the two sides of the active site allowed differentiation between the two substrates. Besides functional optimization the nucleotide identities and conformations evolved for enhancing the stability of the symmetrical region. Consequently, the orientations of a large fraction of the RNA bases of the contemporary symmetrical region violate the internal symmetry (Fig. 4). Thus, with selection pressure for increased stability

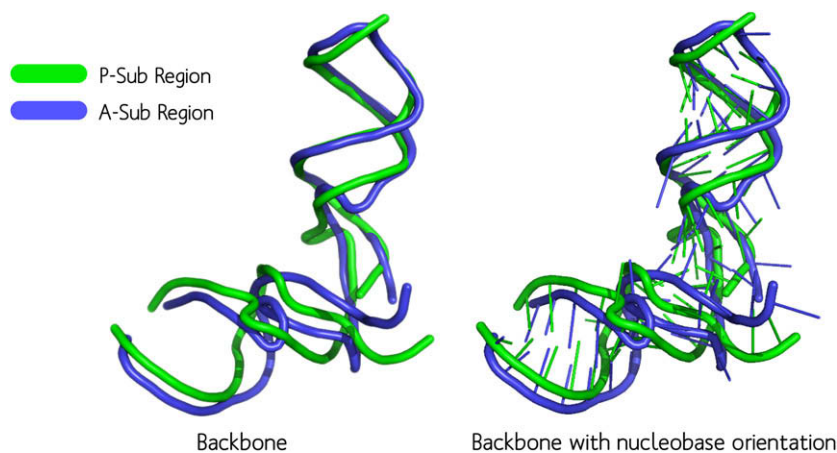


Fig. 4. A feasible route in the optimization of the evolving PTC: superpositions of the rRNA in the A- and P-subregions (in blue and green, respectively). The striking overlap of the backbones of the two subregions, indicating the high level of symmetry, is shown on the left. Deviations from the symmetrical arrangements, indicated by the differences in the orientations of the non base-paired bases of the two subregions, are suggestive of the optimization that each half of the PTC underwent in order to fit the specific task of each site.

and efficiency, the proto-ribosome evolved into an entity that can provide all the activities required for nascent protein elongation in the modern ribosome. As such, the modern ribosome could have evolved gradually around the symmetrical region until it acquired its final shape and could perform programmed translation, either hierarchically [9] or by another mechanism (e.g. [20]).

5. Conclusions

The emergence of Life required an apparatus for synthesizing polypeptides capable of performing catalytic or other life supporting tasks, i.e. the ribosome. The proto-ribosome, which served as the precursor for the modern translation machinery by its capacity to autonomously catalyze peptide bonds forming non-coded amino acid oligo- or polymers, is suggested to have appeared by spontaneous dimeric assembly of two self-folding RNA chains. These pocket-like dimers offered a catalytic site for favorable positioning of the substrates involved in peptide bond formation and simple elongation. Our studies show that it is likely that the proto-ribosome is still embedded in the core of the modern ribosome, and that the tendency for dimerization of the proto-ribosome, a prerequisite for obtaining the catalytic center, is intrinsically linked to the sequences and the folds of its two components, thus indicating functional selection at the molecular level in the prebiotic era.

Acknowledgments

Thanks are due to Ilana Agmon for fruitful discussions and original suggestions; all members of the ribosome group at the Weizmann Institute for continuous interest; Ofir Sade-Falk and Leena Taha for assistance. Support was provided by the US National Inst. of Health (GM34360), and the Kimmelman Center for Macromolecular Assemblies. CD is supported by the Adams Fellowship Program of the Israel Academy of Sciences and Humanities. AY holds the Martin and Helen Kimmel Professorial Chair.

References

- [1] Agmon, I., Bashan, A., Zarivach, R., Yonath, A. (2005) Symmetry at the active site of the ribosome: structural and functional implications. *Biol. Chem.* 386, 833–844.
- [2] Agmon, I., Bashan, A., Yonath, A. (2006) On ribosome conservation and evolution. *Isr. J. Ecol. Evol.* 52, 359–379.
- [3] Agmon, I., Davidovich, C., Bashan, A., Yonath, A. (2009) Identification of the prebiotic translation apparatus within the contemporary ribosome. <http://precedings.nature.com/documents/2921/>.
- [4] Anderson, R.M., Kwon, M., Strobel, S.A. (2007) Toward ribosomal RNA catalytic activity in the absence of protein. *J. Mol. Evol.* 64, 472–483.
- [5] Ban, N., Nissen, P., Hansen, J., Moore, P.B., Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* 289, 905–920.
- [6] Bashan, A., Agmon, I., Zarivach, R., Schluenzen, F., Harms, J., Berisio, R., Bartels, H., Franceschi, F., Auerbach, T., Hansen, H.A.S., Kossoy, E., Kessler, M., Yonath, A. (2003) Structural basis of the ribosomal machinery for peptide bond formation, translocation, and nascent chain progression. *Mol. Cell.* 11, 91–102.
- [7] Bashan, A., Yonath, A. (2008) Correlating ribosome function with high-resolution structures. *Trends Microbiol.* 16, 326–335.
- [8] Bieling, P., Beringer, M., Adio, S., Rodina, M.V. (2006) Peptide bond formation does not involve acid-base catalysis by ribosomal residues. *Nat. Struct. Mol. Biol.* 13, 424–428.
- [9] Bokov, K., Steinberg, S.V. (2009) A hierarchical model for evolution of 23S ribosomal RNA. *Nature* 457, 977–980.
- [10] Davis, J.H., Tonelli, M., Scott, L.G., Jaeger, L., Williamson, J.R., Butcher, S.E. (2005) RNA helical packing in solution: NMR structure of a 30 kDa GAAA tetraloop-receptor complex. *J. Mol. Biol.* 351, 371–382.
- [11] Dick, T.P., Schamel, W.A. (1995) Molecular evolution of transfer RNA from two precursor hairpins: implications for the origin of protein synthesis. *J. Mol. Evol.* 41, 1–9.
- [12] Di Giulio, M. (1992) On the origin of the transfer RNA molecule. *J. Theor. Biol.* 159, 199–214.
- [13] Doudna, J.A., Cech, T.R. (2002) The chemical repertoire of natural ribozymes. *Nature* 418, 222–228.
- [14] Eigen, M., Schuster, P. (1977) The hypercycle. A principle of natural self-organization. Part A: emergence of the hypercycle. *Naturwissenschaften* 64, 541–565.
- [15] Eigen, M., Lindemann, B.F., Tietze, M., Winkler-Oswatitsch, R., Dress, A., von Haeseler, A. (1989) How old is the genetic code? Statistical geometry of tRNA provides an answer. *Science* 244, 673–679.
- [16] Eigen, M. (1993) The origin of genetic information: viruses as models. *Gene* 135, 37–47.
- [17] Ferris, J.P., Montmorillonite. (2002) Catalysis of 30–50 mer oligonucleotides: laboratory demonstration of potential steps in the origin of the RNA world. *Orig. Life Evol. Biosph.* 32, 311–312.
- [18] Fani, R., Fondi, R. (2009) Origin and evolution of metabolic pathways. *Phys. Life Rev.* 6, 23–52.
- [19] Fondi, R., et al, this issue.
- [20] Fox, G.E., Naik, A.K. (2004) The evolutionary history of the ribosome. In L. Ribas de Pouplana (Ed.), *The Genetic Code and the Origin of Life* (pp. 92–105). Landes Bioscience.
- [21] Giel-Pietraszuk, M., Barciszewski, J. (2006) Charging of tRNA with non-natural amino acids at high pressure. *FEBS J.* 273, 3014–3023.
- [22] Gregory, S.T., Dahlberg, A.E. (2004) Peptide bond formation is all about proximity. *Nat. Struct. Mol. Biol.* 11, 586–587.
- [23] Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F., Yonath, A. (2001) High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell* 107, 679–688.
- [24] Holcomb, D.N., Tinoco jun, I. (1965) Conformation of polyriboadenylic acid: pH and temperature dependence. *Biopolymers* 3, 121–133.
- [25] Ilangasekare, M., Sanchez, G., Nickles, T., Yarus, M. (1995) Aminoacyl-RNA synthesis catalyzed by an RNA. *Science* 267, 643–647.
- [26] Jaeger, L., Leontis, N.B. (2000) Tecto-RNA: one-dimensional self-assembly through tertiary interactions. *Angew. Chem. Int. Ed. Engl.* 39, 2521–2524.
- [27] Jeffares, D.C., Poole, A.M., Penny, D. (1998) Relics from the RNA world. *J. Mol. Evol.* 46, 18–36.
- [28] Kholod, N.S. (1999) Dimer formation by tRNAs. *Biochemistry (Mosc)* 64, 298–306.
- [29] Korostelev, A., Trakhanov, S., Laurberg, M., Noller, H.F. (2006) Crystal structure of a 70s ribosome-tRNA complex reveals functional interactions and rearrangements. *Cell* 126, 1065–1077.
- [30] Krzyaniak, A., Barciszewski, J., Salahski, P., Jurczak, J. (1994) The non-enzymatic specific aminoacylation of transfer RNA at high pressure. *Int. J. Biol. Macromol.* 16, 153–158.
- [31] Lehmann, J., Reichel, A., Buguin, A., Libchaber, A. (2008) Efficiency of a self-aminoacylating ribozyme: effect of the length and base-composition of its 3' extension. *RNA* 13, 1191–1197.
- [32] Lincoln, T.A., Joyce, G.F. (2009) Self-sustained replication of an RNA enzyme. *Science* 323, 1229–1232.
- [33] Londei, P. (2005) Evolution of translational initiation: new insights from the archaea. *FEMS Microbiol. Rev.* 29, 185–200.
- [34] Maizels, N., Weiner, A.M. (1994) Phylogeny from function: evidence from the molecular fossil record that tRNA originated in replication, not translation. *Proc. Natl. Acad. Sci. U.S.A.* 91, 6729–6734.

- [35] Mears, J.A., Cannone, J.J., Stagg, S.M., Gutell, R.R., Agrawal, R.K., Harvey, S.C. (2002) Modeling a minimal ribosome based on comparative sequence analysis. *J. Mol. Biol.* 321, 215–234.
- [36] Moore, P.B., Steitz, T.A. (2003) After the ribosome structures: how does peptidyl transferase work? *RNA* 9, 155–159.
- [37] Nagaswamy, U., Fox, G.E. (2003) RNA ligation and the origin of tRNA. *Orig. Life Evol. Biosph.* 33, 199–209.
- [38] Nissen, P., Ippolito, J.A., Ban, N., Moore, P.B., Steitz, T.A. (2001) RNA tertiary interactions in the large ribosomal subunit: the A-minor motif. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4899–4903.
- [39] Orgel, L.E. (1973) *The Origins of Life: Molecules and Natural Selection*. New York: John Wiley and Sons.
- [40] Pino, S., Ciciriello, F., Costanzo, G., Di Mauro, E. (2008) Nonenzymatic RNA ligation in water. *J. Biol. Chem.* 283, 36494–36503.
- [41] Pley, H.W., Flaherty, K.M., McKay, D.B. (1994) Model for an RNA tertiary interaction from the structure of an intermolecular complex between a GAAA tetraloop and an RNA helix. *Nature* 372(1994), 111–113.
- [42] Polacek, N., Mankin, A.S. (2005) The ribosomal peptidyl transferase center: structure, function, evolution, inhibition. *Crit. Rev. Biochem. Mol. Biol.* 40(2005), 285–311.
- [43] Powner, M.W., Gerland, B., Sutherland, J.D. (2009) Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. *Nature* 459, 239–242.
- [44] Roy, M.D., Wittenhagen, L.M., Kelley, S.O. (2005) Structural probing of a pathogenic tRNA dimer. *RNA* 11, 254–260.
- [45] Russell, R. (2008) RNA misfolding and the action of chaperones. *Front Biosci.* 13, 1–20.
- [46] Samanta, D., Mukhopadhyay, D., Chowdhury, S., Ghosh, J., Pal, S., Basu, A., Bhattacharya, A., Das, A., Das, D., DasGupta, C. (2008) Protein folding by domain V of *Escherichia coli* 23S rRNA: specificity of RNA protein interactions. *J. Bacteriol.* 190, 3344–3352.
- [47] Sato, N.S., Hirabayashi, N., Agmon, I., Yonath, A., Suzuki, T. (2006) Comprehensive genetic selection revealed essential bases in the peptidyl-transferase center. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15386–15391.
- [48] Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M., Cate, J.H.D. (2005) Structures of the bacterial ribosome at 3.5 Å resolution. *Science* 310, 827–834.
- [49] Selmer, M., Dunham, C.M., Murphy IV, F.V., Weixlbaumer, A., Petry, S., Kelley, A.C., Weir, J.R., Ramakrishnan, V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* 313, 1935–1942.
- [50] Smith, J.M., Szathmáry, E. (1995) *The Major Transitions in Evolution*. Oxford University Press.
- [51] Smith, T.F., Lee, J.C., Gutell, R.R., Hartman, H. (2008) The origin and evolution of the ribosome. *Biol. Direct.* 3, 16.
- [52] Sun, X., Li, J.M., Wartell, R.M. (2007) Conversion of stable RNA hairpin to a metastable dimer in frozen solution. *RNA* 13, 2277–2286.
- [53] Szostak, J.W. (2009) Systems chemistry on early earth. *Nature* 459, 171–172.
- [54] Talini, G., et al., this issue.
- [55] Voytek, S.B., Joyce, G.F. (2007) Emergence of a fast-reacting ribozyme that is capable of undergoing continuous evolution. *Proc. Natl. Acad. Sci. U.S.A.* 104, 15288–15293.
- [56] Weiner, A.M., Maizels, N. (1987) tRNA-like structures tag the 3' ends of genomic RNA molecules for replication: implications for the origin of protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 84, 7383–7387.
- [57] Weinger, J.S., Parnell, K.M., Dorner, S., Green, R., Strobel, S.A. (2004) Substrate-assisted catalysis of peptide bond formation by the ribosome. *Nat. Struct. Mol. Biol.* 11, 1101–1106.
- [58] Weinger, J.S., Strobel, S.A. (2007) Exploring the mechanism of protein synthesis with modified substrates and novel intermediate mimics. *Blood Cells Mol. Dis.* 38, 110–116.
- [59] Weixlbaumer, A., Jin, H., Neubauer, C., Voorhees, R.M., Petry, S., Kelley, A.C., Ramakrishnan, V. (2008) Insights into translational termination from the structure of RF2 bound to the ribosome. *Science* 322, 953–956.
- [60] Woese, C.R. (1972) *The Emergence of Genetic Organization*. North-Holland Publishing. pp. 302–332.
- [61] Woese, C.R. (1973) Evolution of the genetic code. *Naturwissenschaften* 60, 447–459.
- [62] Woese, C.R. (2001) Translation: in retrospect and prospect. *RNA* 7, 1055–1067.
- [63] Yarus, M. (2002) Primordial genetics: phenotype of the ribocyte. *Annu. Rev. Genet.* 36, 125–151.
- [64] Yonath, A. (2003) Ribosomal tolerance and peptide bond formation. *Biol. Chem.* 384, 1411–1419.
- [65] Youngman, E.M., Brunelle, J.L., Kochaniak, A.B., Green, R. (2004) The active site of the ribosome is composed of two layers of conserved nucleotides with distinct roles in peptide bond formation and peptide release. *Cell* 117, 589–599.