Biochemical Society Transactions (2010) Volume 38, part 2

Ancient machinery embedded in the contemporary ribosome

Matthew J. Belousoff*, Chen Davidovich*, Ella Zimmerman*, Yaron Caspi*[1], Itai Wekselman*, Lin Rozenszajn*[2],Tal Shapira*, Ofir Sade-Falk*[3], Leena Taha*[4], Anat Bashan*, Manfred S. Weiss† and Ada Yonath*[5]

*Department of Structural Biology, Weizmann Institute, 2 Herzl Street, Rehovot, 76100 Israel, and †EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85,
D-22603 Hamburg, Germany

Ancient machinery embedded in the contemporary ribosome

Matthew J. Belousoff*, Chen Davidovich*, Ella Zimmerman*, Yaron Caspi*[1], Itai Wekselman*, Lin Rozenszajn*[2], Tal Shapira*, Ofir Sade-Falk*[3], Leena Taha*[4], Anat Bashan*, Manfred S. Weiss† and Ada Yonath*[5]

*Department of Structural Biology, Weizmann Institute, 2 Herzl Street, Rehovot, 76100 Israel, and †EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany

Abstract

Structural analysis, supported by biochemical, mutagenesis and computational evidence, indicates that the peptidyltransferase centre of the contemporary ribosome is a universal symmetrical pocket composed solely of rRNA. This pocket seems to be a relic of the proto-ribosome, an ancient ribozyme, which was a dimeric RNA assembly formed from self-folded RNA chains of identical, similar or different sequences. This could have occurred spontaneously by gene duplication or gene fusion. This pocket-like entity was capable of autonomously catalysing various reactions, including peptide bond formation and non-coded or semi-coded amino acid polymerization. Efforts toward the structural definition of the early entity capable of genetic decoding involve the crystallization of the small ribosomal subunit of a bacterial organism harbouring a single functional rRNA operon.

Introduction

The ribosome is the universal naturally occurring ribozyme that performs translation of the genetic code into nascent proteins. These cellular catalytic machines are multi-component riboproteins of molecular mass 2.5 and 4 MDa (for prokaryotic and eukaryotic sources respectively). Remarkably, despite the size difference, the functional regions of ribosomes, the decoding centre and the PTC (peptidyltransferase centre) composed solely of rRNA, are highly conserved across all domains of life.

Involvement of RNA-rich particles in genetic expression was suggested over five decades ago [1,2], when RNA-rich 'Palade particles' were observed in cells, in close proximity to the endoplasmic reticulum membrane [1,3]. The discovery of the ribosomes and the realization of their complexity stimulated attempts to define their precursors, by comparative sequence analogy [4], *in vitro* selection [5], functional and mutagenesis investigations [6,7], and analyses of the nature of internal ribosome interactions [8–10]. These studies illuminated key ribosomal properties and stimulated hypotheses on the way the ribosome developed, but did not reveal the entity that could be assigned as the proto-ribosome.

In the present paper, we present arguments supporting the existence of ancestral dimeric entity that may have acted as machinery capable of catalysing various reactions, including peptide bond formation.

Peptide bonds are formed in a symmetrical region within the asymmetric ribosome

The heart of the contemporary ribosome hosts the PTC, i.e. the site of peptide bond formation. This centre is located in the midst of a symmetrical 'pocket-like' structure [11–17] (Figure 1) that encompasses 180 nucleotides of which the backbone folds, irrespective of the nucleotide sequences, are related by an internal pseudo-2-fold symmetry. This is an extremely unusual feature, as the ribosome, composed of over 4500 nucleotides and over 50 proteins, is highly asymmetric. This internal symmetry exists in all known high-resolution ribosome structures [18–22] and its exceptionally high sequence conservation indicates its ancient origin.

The architecture of this structural element provides the framework for all of the ribosome's catalytic contributions towards peptide bond formation, since it positions the ribosome's substrates in favourable stereochemistry for peptide bond formation and substrate-mediated catalysis. Furthermore, by encircling the PTC, the symmetrical region confines the void required for the motions involved in the translocation of the 3′-end of the aminoacylated tRNA from the A-site into the site of the peptidylated tRNA molecules (P-site). Hence the symmetrical region also provides the mechanism facilitating the polymerase activity of the ribosome [11,13,14].

On the basis of the detection of the internal pseudo-symmetry within the ribosomal catalytic region, we have proposed that, as in modern life, the ancient machine for forming peptide bonds was made exclusively from RNA. It is widely accepted that such RNA oligomers could have formed

Key words: evolution, *Mycobacterium smegmatis*, proto-ribosome, ribosome, symmetrical region.

Abbreviations used: PTC, peptidyltransferase centre.

[1]**Present address:** Harvard FAS Center for Systems Biology, 52 Oxford Street, Cambridge, MA 02138, U.S.A.

[2]**Present address:** University of Toronto, 27 King's College Circle, Toronto, Ontario, Canada, M5S 1A1.

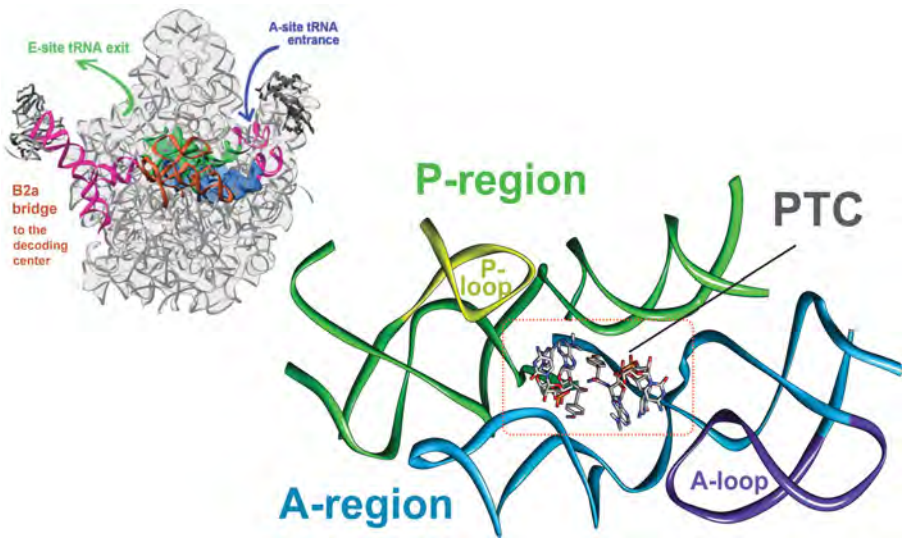
[3]**Present address:** Department of Biological Chemistry, Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel.

[4]**Present address:** Hebrew University of Jerusalem, Jerusalem 91904, Israel.

[5]**To whom correspondence should be addressed (email Ada.Yonath@weizmann.ac.il)**

Figure 1 | **The symmetrical region of the 50S ribosome**

The RNA backbone of the two symmetrical components: the A-region is shown in blue and dark blue, and the P-site is shown in green and olive-green. The PTC (orange box) is occupied by two substrates mimicking the tRNA 3′-end. Inset: the interface view of the large ribosomal subunit (grey), indicating the functional regions connected directly to the symmetrical region (in blue and green).



in the primordial soup [23–25] by various mechanisms, including chemical (non-enzymatic) RNA synthesis and polymerization under conditions mimicking those assumed to govern the prebiotic era [26–28].

It appears that the ancient short RNA chains acquired common motifs that were sufficiently stable to survive environmental alterations and evolutionary stress. Such motifs have been identified in various natural ribozymes, some of which are believed to be relics from the prebiotic world. These include gene regulators, riboswitches, RNA polymerases and ribozymes catalysing phosphodiester cleavage, RNA processing and RNA modification [29].

Emergence of the proto-ribosome

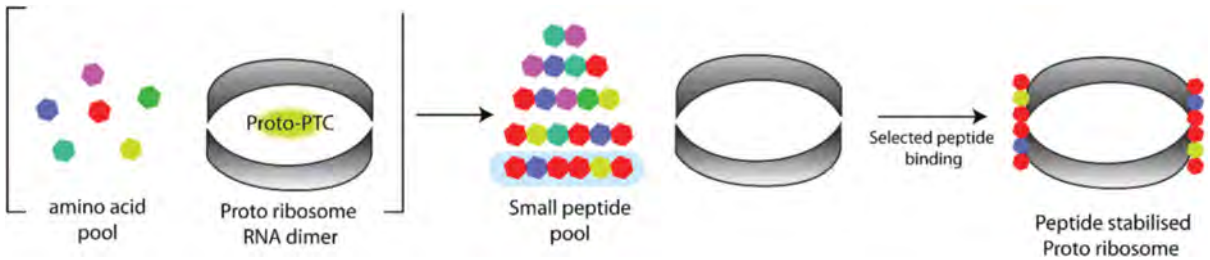
Among the existing RNA chains, those forming entities that could later evolve to become the precursor of the proto-ribosome underwent self dimerization and acquired a ‘pocket-like’ configuration, capable of hosting variety of substrates. Subsequently, RNA chains of lengths and compositions sufficient for the construction of basic elements of the modern ribosome active site may have existed in the prebiotic period, becoming like pocket-like molecular machines with either non-specific or specialized catalytic capabilities [30,31].

Dimerization in a symmetrical manner could have occurred spontaneously, utilizing chemical complementarity obtained by tertiary interactions, such as the common GNRA (guanine–any nucleotide–a purine–adenine) system [32] that includes the abundant and ubiquitous ‘A-minor’ structural motif [9,33,34]. This seemingly simple process could be assisted by other molecules acting as small chaperones [35] offering stabilization. Additional structural support could be obtained from short peptides that have high affinity for RNA [36], or from longer poly-amino acids, similar to protein–RNA interactions within the contemporary ribosome, or from interactions of low-molecular-mass compounds with exposed regions of RNA. These short peptides could have been produced by uncoded elongation by early versions of the proto-ribosome or by other means. Following this mechanism, a proto-ribosome that produced peptides with amino acid compositions that provided with better fitness, thus had higher probability of being retained.

On the basis of the presumed ability of the prebiotic construct to enable peptide bond formation, it is suggested that the symmetrical region of the contemporary ribosome originated from a symmetrical proto-ribosome [14,15,17,37]. Its substrates could have been activated amino acids or spontaneously produced amino acids conjugated with single or short oligonucleotides [38–41].

What could have been the driving force for the construction of a machine that forms peptide bonds in the RNA world? It is conceivable that a pocket-like entity existed in the RNA world for performing required chemical reactions, involving mainly RNA molecules. An examples could be a primitive RNA replisome, an entity that was proposed to be the ancestor of the ribosome [42] and could perform RNA polymerization and/or simple RNA replication. Similarly, the original ancient machines could have been an integral part of the RNA metabolic pathways [43] within some sort of a proto-cell. Regardless of the tasks of these hypothetical

Figure 2 | **Generation of a pool of peptides by the proto-ribosome (RNA represented as grey curves), out of which a small portion is selected for RNA binding, stabilizing the proto-ribosome and enhancing its peptidyltransferase activity**



ancient molecular machines, it appears that polymerization of amino acids became the task of highest preference. Consequently, stable pocket-like molecular dimers capable of peptide bond formation were evolutionarily favoured, surviving under various environmental conditions, and later becoming the ancestor of the active site of the contemporary ribosome. As it is assumed that, in the prebiotic era, RNA chains could self-replicate [44–48], it is conceivable that phenotypes with favourable properties could have been synthesized in many copies. It is therefore likely that some of these phenotypes originated by the fusion of two different or duplication of two identical sequences.

We assessed the feasibility of a dimeric proto-ribosome capable of peptide bond formation biochemically, by testing the tendency of various RNA chains to dimerize and to form peptide bonds. Among the various RNA sequences that have been constructed, several, but 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 [15]. Specifically, so far, a marked preference was detected for the dimerization of sequences resembling the PTC P-site, including those that underwent mutational alterations by *in vitro* site-directed mutagenesis. This noticeable preference may indicate higher stability of this portion of the symmetrical region, or a higher significance in the process of peptide bond formation, in accordance with the contemporary accommodation of the initial tRNA at the P-site of the PTC.

Emergence of coded translation

What would have been the selective benefit for a ribozyme functioning in the RNA world to begin the process of amino acid ligation? It is clear *a posteriori* that proteins are much better catalysts than their ribozyme cousins. However, evolution has no ability to discern future functionality from its present state. Accordingly, there must have been an immediate reason for the production of short 'uncoded' peptides by an emerging proto-ribosome.

It has been postulated that, among the first amino acids to be incorporated into a primeval genetic code, were the amino acids with the highest 'catalytic potential' [49]. This hypothesis yields insight into what type of amino acids would have been preferentially elongated by the proto-ribosome, as they presumably gave enough selection advantage to warrant the formation of their coded elongation. Encouragingly, these same amino acids (histidine, lysine, arginine) are the ones that interact strongly with RNA in extant biology.

It is conceivable, then, that the proto-ribosome would have initially favoured peptidyl transfer and elongation of histidine, lysine and arginine because of the favourable electrostatic and hydrogen-bonding interactions that these amino acids exhibit with RNA. Upon elongation by the proto-ribosome, a pool of short peptides would have been generated, some of which would be rich in the amino acids that can be attracted to the negatively charged RNA. Plausibly, a small portion of this peptide pool would have displayed the ability to bind to the ribozyme that produced them. Of these RNA-binding peptides, some would have stabilized further the RNA structure, or caused it to adopt an even more active conformation for peptidyl transfer (Figure 2).

Simultaneously, the small peptide pool generated by a proto-ribosome must have had the ability to assist a portion of the RNA machinery that was in the ribocyte (a postulated ancient RNA-based cell), as observed in modern ribozymes that are supported by protein assemblies that enhance their reactivity (i.e. group I intron ribozyme) [50]. Hence a proto-ribosome that could manufacture a set of small peptides that stabilized its own function and/or assist the other surrounding ribozymes would have imparted a significant selection advantage.

Clearly, in modern life, only a small portion of proteins functionally interact with RNA, whereas the majority of them are self-sufficient in their function. Modern enzymes adopt highly specific tertiary structures to achieve their high rates of catalysis, but these complicated folded structures would not have been available to the emerging proto-peptide pool. Probably, the preferentially elongated amino acids were the positively charged histidine, arginine and lysine, which are also rich in chemical reactivity. Although, presumably, these peptides were unfolded (or largely unstructured), those rich in these moieties could have enriched their neighbourhood and served as the first non-RNA catalysts [16]. For example, imidazole rings (of histidine) can act as a proton-transfer agent and nucleophilic catalyst [51],

guanidine (found in arginine) can stabilize transitions states by favourable hydrogen-bonding interactions [52,53], and the primary amine in lysine can act as general acid–base catalyst. Although the reaction rates of these small peptide catalysts would have been incomparable with modern enzymatic catalysts. The RNA world was a 'slow-living entity' [48], so the addition of any new chemical functionality would have been vital to add selection advantage to this primordial life.

The transition from a molecule forming peptide bonds to an elaborate apparatus capable of decoding genetic information was coupled with the evolving genetic code and the development of the substrates. A higher level of efficiency could have been achieved by the increase of the proto-ribosome size and the creation of a supporting environment coupled with the elongation of the substrates forming the minimal mono- or oligo-nucleotide–amino acid conjugates towards the modern tRNA. The conversion into longer compounds with a contour that can complement the inner surface of a larger reaction pocket occurred concurrently with proto-ribosome mutational optimization, aimed at accurate substrate positioning that, in the modern ribosome, is governed by remote interactions beyond the PTC [11,54].

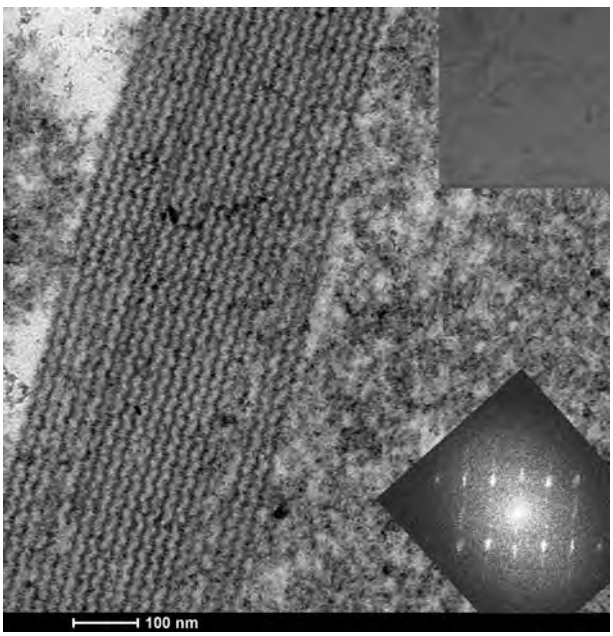
Later stages of this development were coupled with the advance from non- or pseudo-genetic peptide bond formation towards performing genetically driven translation and with the optimization of selected portions of the evolving machine for their specific tasks. Additionally recruited structural elements, which could be oligopeptides or oligonucleotides, interacted with the proto-ribosome and its surroundings in a manner resembling the protein–RNA interactions in the modern ribosome. 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, orientations of the RNA bases within the contemporary symmetrical region are not related by the internal symmetry, consistent with the finding 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. With the selection pressure for increased stability and efficiency, the proto-ribosome served as the entity providing all the activities required for non-coded nascent protein elongation. The modern ribosome could have evolved gradually around the symmetrical region until it acquired its final shape, either hierarchically [9] or by another mechanism (e.g. [55]).

Future prospects

The growing complexity of the ribosome in size and shape allowed it to perform programmed translation. Encouraged by the identification of the ancient peptide bond formation machinery within the large ribosomal subunit, we searched for remnants of a primitive decoding region within the small subunit. Because decoding does not require symmetry or any other distinct structural motif, unambiguous identification of the ancestor region was so far not possible. We therefore selected the small ribosomal subunit from *Mycobacterium smegmatis*, a strain of a bacterial organism that was genetically manipulated to harbour a single functional rRNA operon [56], in order to approach this issue using mutagenesis combined by X-ray crystallography. The microcrystals (Figure 3) obtained so far indicated feasibility of crystallographic studies, as they are of a quality comparable with the first ribosomal crystals, obtained almost 30 years ago [57], which could be improved to yield high-resolution structures.

Figure 3 | Electron micrograph of microcrystals of the 30S ribosomal subunit from *M. smegmatis*

Insets: top, light photomicrograph of microcrystals; bottom, fast Fourier transform (FFT) of the electron micrographs.



Acknowledgments

Thanks are due to all members of the ribosome group at the Weizmann Institute for continuous interest, Shosh Tel-Or and Donna Matzov for technical assistance, the electron microscope unit, especially Sharon Wolf and Helena Sabanay, for providing experimental support and to Dr Erik Bottger for encouraging the *M. smegmatis* crystallization. Crystallographic data were collected at ID23-2, European Synchrotron Radiation Facility, Grenoble, France.

Funding

Support was provided by the National Institutes of Health [grant number GM34360], the German–Israeli Foundation [grant number

G-853-284.9/2004] and the Kimmelman Centre for Macromolecular Assemblies. C.D. is supported by the Adams Fellowship Program of the Israel Academy of Sciences and Humanities. A.Y. holds the Martin and Helen Kimmel Professorial Chair.

References

1 Watson, J.D. (1963) Involvement of RNA in the synthesis of proteins. Science **140**, 17–26
2 Crick, F.H. (1968) The origin of the genetic code. J. Mol. Biol. **38**, 367–379
3 Palade, G.E. (1955) A small particulate component of the cytoplasm. J. Biophys. Biochem. Cytol. **1**, 59–68
4 Mears, J.A., Cannone, J.J., Stagg, S.M., Gutell, R.R., Agrawal, R.K. and Harvey, S.C. (2002) Modeling a minimal ribosome based on comparative sequence analysis. J. Mol. Biol. **321**, 215–234
5 Anderson, R.M., Kwon, M. and Strobel, S.A. (2007) Toward ribosomal RNA catalytic activity in the absence of protein. J. Mol. Evol. **64**, 472–483
6 Polacek, N. and Mankin, A.S. (2005) The ribosomal peptidyl transferase center: structure, function, evolution, inhibition. Crit. Rev. Biochem. Mol. Biol. **40**, 285–311
7 Sato, N.S., Hirabayashi, N., Agmon, I., Yonath, A. and Suzuki, T. (2006) Comprehensive genetic selection revealed essential bases in the peptidyl-transferase center. Proc. Natl. Acad. Sci. U.S.A. **103**, 15386–15391
8 Smith, T.F., Lee, J.C., Gutell, R.R. and Hartman, H. (2008) The origin and evolution of the ribosome. Biol. Direct **3**, 16
9 Bokov, K. and Steinberg, S.V. (2009) A hierarchical model for evolution of 23S ribosomal RNA. Nature **457**, 977–980
10 Hsiao, C., Mohan, S., Kalahar, B.K. and Williams, L.D. (2009) Peeling the onion: ribosomes are ancient molecular fossils. Mol. Biol. Evol. **23**, 2415–2425
11 Bashan, A., Agmon, I., Zarivach, R., Schluenzen, F., Harms, J., Berisio, R., Bartels, H., Franceschi, F., Auerbach, T., Hansen, H.A.S. et al. (2003) Structural basis of the ribosomal machinery for peptide bond formation, translocation, and nascent chain progression. Mol. Cell **11**, 91–102
12 Bashan, A., Belousoff, M.J., Davidovich, C. and Yonath, A. (2009) Linking the RNA world to modern life: the proto-ribosome conception. Origins Life Evol. Biosphere, in the press
13 Agmon, I., Bashan, A., Zarivach, R. and Yonath, A. (2005) Symmetry at the active site of the ribosome: structural and functional implications. Biol. Chem. **386**, 833–844
14 Agmon, I., Bashan, A. and Yonath, A. (2006) On ribosome conservation and evolution. Isr. J. Ecol. Evol. **52**, 359–379
15 Davidovich, C., Belousoff, M., Bashan, A. and Yonath, A. (2009) The evolving ribosome: from non-coded peptide bond formation to sophisticated translation machinery. Res. Microbiol. **160**, 487–492
16 Belousoff, M.J., Davidovich, C., Bashan, A. and Yonath, A. (2009) On the development towards the modern world: a plausible role of uncoded peptides in the RNA world. Origins Life Evol. Biosphere
17 Yonath, A. (2009) Large facilities and the evolving ribosome, the cellular machine for genetic-code translation. J. R. Soc. Interface **6** (Suppl. 5), S575–S585
18 Ban, N., Nissen, P., Hansen, J., Moore, P.B. and Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science **289**, 905–920
19 Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F. and Yonath, A. (2001) High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. Cell **107**, 679–688
20 Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M. and Cate, J.H.D. (2005) Structures of the bacterial ribosome at 3.5 Å resolution. Science **310**, 827–834
21 Korostelev, A., Trakhanov, S., Laurberg, M. and Noller, H.F. (2006) Crystal Structure of a 70S ribosome–tRNA complex reveals functional interactions and rearrangements. Cell **126**, 1065–1077
22 Selmer, M., Dunham, C.M., Murphy, IV, F.V., Weixlbaumer, A., Petry, S., Kelley, A.C., Weir, J.R. and Ramakrishnan, V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. Science **313**, 1935–1942
23 Jaeger, L. and Leontis, N.B. (2000) Tecto-RNA: one-dimensional self-assembly through tertiary interactions. Angew. Chem. Int. Ed. Engl. **39**, 2521–2524
24 Jeffares, D.C., Poole, A.M. and Penny, D. (1998) Relics from the RNA world. J. Mol. Evol. **46**, 18–36
25 Orgel, L.E. (1973) The Origins of Life: Molecules and Natural Selection, John Wiley and Sons, New York
26 Powner, M.W., Gerland, B. and Sutherland, J.D. (2009) Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature **459**, 239–242
27 Szostak, J.W. (2009) Origins of life: systems chemistry on early Earth. Nature **459**, 171–172
28 Pino, S., Ciciriello, F., Costanzo, G. and Di Mauro, E. (2008) Nonenzymatic RNA ligation in water. J. Biol. Chem. **283**, 36494–36503
29 Doudna, J.A. and Cech, T.R. (2002) The chemical repertoire of natural ribozymes. Nature **418**, 222–228
30 Eigen, M., Lindemann, B.F., Tietze, M., Winkler-Oswatitsch, R., Dress, A. and von Haeseler, A. (1989) How old is the genetic code? Statistical geometry of tRNA provides an answer. Science **244**, 673–679
31 Ferris, J.P. (2002) Montmorillonite catalysis of 30–50 mer oligonucleotides: laboratory demonstration of potential steps in the origin of the RNA world. Origins Life Evol. Biosphere **32**, 311–332
32 Pley, H.W., Flaherty, K.M. and 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**, 111–113
33 Davis, J.H., Tonelli, M., Scott, L.G., Jaeger, L., Williamson, J.R. and 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
34 Nissen, P., Ippolito, J.A., Ban, N., Moore, P.B. and 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
35 Russell, R. (2008) RNA misfolding and the action of chaperones. Front. Biosci. **13**, 1–20
36 Llano-Sotelo, B., Klepacki, D. and Mankin, A.S. (2009) Selection of small peptides, inhibitors of translation. J. Mol. Biol. **391**, 813–819
37 Bashan, A. and Yonath, A. (2008) Correlating ribosome function with high-resolution structures. Trends Microbiol. **16**, 326–335
38 Illangasekare, M., Sanchez, G., Nickles, T. and Yarus, M. (1995) Aminoacyl-RNA synthesis catalyzed by an RNA. Science **267**, 643–647
39 Giel-Pietraszuk, M. and Barciszewski, J. (2006) Charging of tRNA with non-natural amino acids at high pressure. FEBS J. **273**, 3014–3023
40 Krzyzaniak, A., Barciszewski, J., Salanski, P. and Jurczak, J. (1994) The non-enzymatic specific amino-acylation of transfer RNA at high pressure. Int. J. Biol. Macromol. **16**, 153–158
41 Lehmann, J., Reichel, A., Buguin, A. and Libchaber, A. (2007) Efficiency of a self-aminoacylating ribozyme: effect of the length and base-composition of its 3′ extension. RNA **13**, 1191–1197
42 Campbell, J.H. (1991) An RNA replisome as the ancestor of the ribosome. J. Mol. Evol. **32**, 3–5
43 Fani, R. and Fondia, M. (2009) Origin and evolution of metabolic pathways. Phys. Life Rev. **6**, 23–52
44 Eigen, M. (1993) The origin of genetic information: viruses as models. Gene **135**, 37–47
45 Smith, J.M. and Szathmáry, E. (1995) The Major Transitions in Evolution, Oxford University Press, Oxford
46 Lincoln, T.A. and Joyce, G.F. (2009) Self-sustained replication of an RNA enzyme. Science **323**, 1229–1232
47 Woese, C.R. (2001) Translation: in retrospect and prospect. RNA **7**, 1055–1067
48 Yarus, M. (2002) Primordial genetics: phenotype of the ribocyte. Annu. Rev. Genet. **36**, 125–151
49 Kun, A., Pongor, S., Jordán, F. and Szathmáry, E. (2008) Catalytic propensity of amino acids and the origins of the genetic code and proteins. in The Codes of Life: the Rules of Macroevolution, vol. 1 (Barbieri, M., ed.), pp. 39–58, Springer, Berlin
50 Stahley, M.R. and Strobel, S.A. (2005) Structural evidence for a two-metal-ion mechanism of group I intron splicing. Science **309**, 1587–1590
51 Roth, A. and Breaker, R.R. (1998) An amino acid as a cofactor for a catalytic polynucleotide. Proc. Natl. Acad. Sci. U.S.A. **95**, 6027–6031
52 Takada, K., Tanaka, S. and Nagasawa, K. (2009) Asymmetric Mannich-type reaction of aromatic α-amido sulfone with malonate using guanidine–thiourea bifunctional organocatalyst. Synlett **10**, 1643–1646
53 Ube, H. and Terada, M. (2009) Enantioselective Henry (nitroaldol) reaction catalyzed by axially chiral guanidines. Bioorg. Med. Chem. Lett. **19**, 3895–3898
54 Yonath, A. (2003) Ribosomal tolerance and peptide bond formation. Biol. Chem. **384**, 1411–1419

55 Fox, G.E. and Naik, A.K. (2004) The evolutionary history of the ribosome. in The Genetic Code and the Origin of Life (Ribas de Poplana, L., ed.), pp. 92–105, Landes Bioscience, Austin
56 Sander, P., Prammananan, T. and Bottger, E.C. (1996) Introducing mutations into a chromosomal rRNA gene using a genetically modified eubacterial host with a single rRNA operon. Mol. Microbiol. **22**, 841–848
57 Yonath, A., Muessig, J., Tesche, B., Lorenz, S., Erdmann, V.A. and Wittmann, H.G. (1980) Crystallization of the large ribosomal subunit from *B. stearothermophilus*. Biochem. Int. **1**, 315–428

Received 16 August 2009
doi:10.1042/BST0380422