molecular self-organization (sometimes called "chemical evolution") to the existing laws of chemistry and physics, both on Earth and elsewhere in the universe.

References

Feinberg G, Shapiro R (1980) Life Beyond Earth. Morrow, New York
Kauffman S (1994) At Home in the Universe. Oxford University Press, New York
Morowitz HJ (1968) Energy Flow in Biology. Academic Press, New York
Morowitz HJ (1999) A theory of biochemical organization, metabolic pathways, and evolution. Complexity 4:39–53
Orgel LE (2008) The Implausibility of metabolic cycles on the Prebiotic Earth. PloS Biol 6: 5–13
Pross A (2004) Causation and the origin of life: metabolism or replication first? Orig Life Evol Biosph 34:307–321
Shapiro R (2000) A replicator was not involved in the origin of life. IUBMB Life 49:173–176
Shapiro R (2006) Small molecule interactions were central to the origin of life. Quart Rev Biol 81:105–125
Spiegelman S, Haruna I, Holland, IB, Beaudreau G, Mills D (1965) The synthesis of a self-propagating and infectious nucleic Acid with a purified enzyme. Proc Natl Acad Sci USA 543:919–927
Wächtershäuser G (1990) Evolution of the first metabolic cycles. Proc Natl Acad Sci USA 87:200–204

E-mail: rs2@nyu.edu

On the Development Towards the Modern World: A Plausible Role of Uncoded Peptides in the RNA World

Matthew J. Belousoff, Chen Davidovich, Anat Bashan, Ada Yonath

Dept of Structural Biology, Weizmann Institute, Israel

Keywords Proto-ribosome • Peptide Bond Formation • Uncoded Peptides • Genetic-code Evolution

Arguably one of the most outstanding problems in understanding the progress of early life is the transition from the RNA world to the modern protein based world. One of the main requirements of this transition is the emergence of mechanism to produce functionally meaningful peptides and later, proteins. What could have served as the driving force for the production of peptides and what would have been their properties and purpose in the RNA world? The answer may seem immediately clear; proteins are better enzymes than ribozymes. However, modern proteins are only useful in their folded stated, whereas peptides need to reach a critical size and specific amino acid sequence before they fold into a functional biomolecules. It is more than likely that emerging peptides were not immediately useful as operational enzymes. Herein we describe two plausible roles that emerging peptides could have played, firstly support of the pre-existing RNA machinery and secondly as early chemical catalysts.

During the era of the RNA world, protein (or peptide) evolution would have been strongly coupled to the extant RNA infrastructure development. Thus, the emergence of peptides as a mechanism for supporting pre-existing RNA machinery, is a sound reason for peptides to be retained in an RNA world. In this issue we briefly discuss ideas for the apparatus that would have served as the proto-ribosome (See Bashan et al. this volume), hypothesized to be a symmetrical 'pocket-like' RNA dimer capable of simple peptidyl

transfer and elongation, in essence non-coded peptide production (Agmon, et al. 2006, Agmon et al. 2005; Bashan et al. 2003).

What potential role would these early peptides have played? Some clues may surface from examination of ribosomal proteins in the structure of modern ribosomes, (Figure 1) where r-proteins are located at the interfaces of A-minor RNA-RNA interactions, namely Adenine and the minor groove of an RNA double helix (Nissen et al. 2001). It has been proposed that the bulk of the rRNA evolved *via* extensions of such A-minor interactions (Bokov et al. 2009) and it is more than likely that the initial proto-ribosome dimer was held together by similar RNA-RNA interactions. However, these interactions are mediated by only 4–6 hydrogen bonds, hence, for efficient function under changing environmental conditions over a significantly long period additional support can be advantageous. In the example of L2 and L36 in the 50S subunit of *D. Radiodurans*, the bulk of the RNA-protein interactions are mediated by positively charged residues. Interestingly, as with most ribosomal proteins, L2 and L36 are lysine, arginine and histidine rich, likely due to their greater propensity to form favorable complimentarity between their positive charge and the negatively charged phosphate ester backbone.

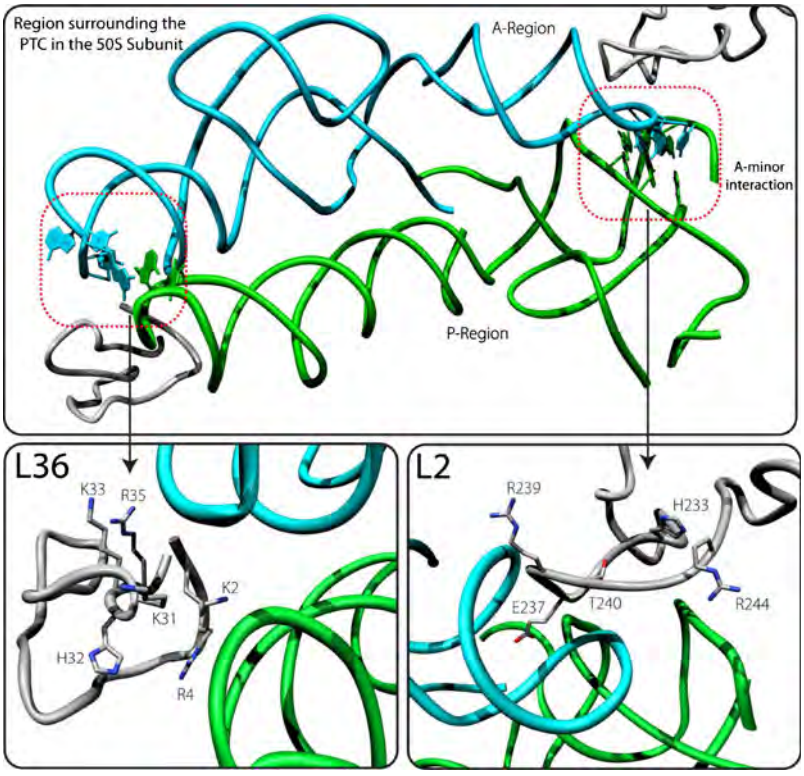
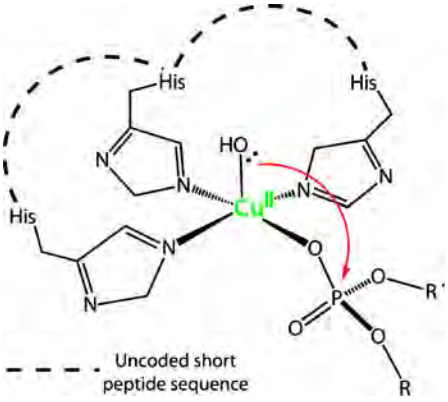


Fig. 1 A portion of the rRNA and proteins surrounding the peptidyl transferase centre in the 50S ribosomal subunit of *Deinococcus Radiodurans*, highlighting the stabilization of the A-minor interactions (in red box) by rProteins L2 and L36. Shown in the lower boxes is the RNA stabilized by positively charged amino acids side chains. The blue and green ribbons indicate the RNA backbone, the grey ribbons indicate the C-alpha positions of the proteins, with selected sidechains visible (PDB entry: 2ZJR) (Harms et al. 2008)

These clues on stabilizing role that ribo-proteins play, may indicate the driving force for the production of small peptides in the RNA world in accord with the observation that even a small arginine rich peptide portion of the Tat protein (a HIV-1 regulatory protein), bind with high selectivity and affinity with its cognate RNA partner, the transcription activation response element (TAR) (Sannes-Lowery et al. 1997).

In a world where replication, metabolism and chemistry is governed by RNA, one of the survival traits that emerging peptides must have exploited was binding to the agents of their production. The amino acids most likely to fulfill this role of RNA binding are ones that are positively charged (*e.g.* histidine, lysine and arginine), those heavily involved in modern RNA binding motifs. Furthermore, it has been postulated that it is these amino-acids that have the a 'higher catalytic propensity' (Kun et al. 2007), making them the ideal candidates for a dual role entities, either as naked peptides or as RNA-peptide hybrids. These types of amino-acids bear chemical functionality that makes them rich in reactivity. For example the imidazole ring in histidine can act as proton transfer agent, as well as a nucleophilic catalyst (Roth et al. 1998). Similarly, the guanidine in arginine has the ability to stabilize transition states of certain reactions such as phosphate ester cleavage (Kim et al. 1991). Conceivably, even short unfolded peptides rich in 'lysine', 'histidine' and 'arginine' could have served as simple catalysts or co-factors (Kun et al. 2007), yielding a selection advantage over peptides without these amino-acids. In support of this claim it has been shown that 'random' oligomers and polymers containing imidazole have catalytic properties for a range of chemical reactions (Okhapkin et al. 2004; Wulff 2002).

A mechanism that could have 'super-charged' the catalytic propensity of these proto-peptides would have been the formation of transition metal complexes involved in a larger range of chemical functionality and catalysis. For example short peptides, such as minimized constructs from avian prions and Amyloid-β peptides bind Cu(II) and Zn(II) ions with high affinity (Hornshaw et al. 1995; Syme et al. 2004), and Cu(II) and Zn(II) complexes that are coordinated by 'histidine' like moieties are fully functional catalysts for simple reactions such as manipulation of phosphate esters (Belousoff et al. 2008; Young et al. 1995) what would have been beneficial in an RNA world. Out of the pool of histidine rich peptides, it is possible that a certain amount could adopt a geometry enabling accommodation of transition metal ions, thus forming an 'active' site. A proposed example of a metallo-proto-peptide catalyst is shown in scheme 1, where a Cu(II) bound histidine rich peptide conjugate would be able to catalyze the hydrolysis of phosphate esters.



Scheme 1 Proto-peptide-metal complex as an early catalyst. A Copper(II)-histidine complex that could act as a potential phosphatase

We therefore propose that during the stage of uncoded translation preference for elongation may have been given to amino acids that were chemically compatible with the ribozyme reacting with them, as well as with other ribozymes and components in its immediate surroundings. Thus, suggesting preferred elongation of combinations of histidines, lysines and arginines in preference to other amino acids. A possible mechanism for such preference of the positively charged residues by the proto-ribosome could be the result of both substrate binding and product release. Positively charged amino acids were likely to fit well within the electrostatic field of the negatively charged RNA-made binding pocket. On the same principle, short peptides with large portion of positively charged amino acids could have spent more time in its surroundings before termination occurred. As at initial stages of the proto-ribosome evolution termination was likely to be a stochastic process, positively charged peptides probably had higher probability to be longer than neutral or acidic chains. Interestingly, phylogenetic analysis indicated that many of the last universal common ancestor (LUCA) peptide motifs are embedded within modern proteins involved in either RNA processing or nucleotide binding (Sobolevsky et al. 2007; Trifonov 2009; Trifonov et al. 2009). Moreover, LUCA motifs consistently contain a GKT peptide combination, supporting potential preference for that these amino acids (*i.e.* H, K, R) were the first to be incorporated into an early genetic code for coded protein production (Kun et al. 2007).

While the exact nature of the crossover from uncoded peptide elongation to the emergence of an amino-acid coding system is shrouded in mystery, evidence about how proteins interact with RNA in modern life can yield clues as to what sort of peptides were selected in an uncoded RNA world. If certain peptides were chemically selected and elongated, the pool of biological molecules available for the ribozymes to interact with becomes enriched with a smaller subset of amino acids or small peptides, concurrently opening the door for primitive coding mechanisms (such as coding coenzyme handles, Szathmary 1993) to emerge in the RNA world as well as providing rudimentary catalysts.

Acknowledgments
We thank all members of the ribosome group at the Weizmann Institute for continuous interest for fruitful discussions. 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

Agmon I, Bashan A, Zarivach R, Yonath A (2005) Symmetry at the active site of the ribosome: structural and functional implications. Biol Chem 386(9):833–844

Agmon I, Bashan A, Yonath A (2006) On ribosome conservation and evolution. Israel J Ecol Evol 52:359–374

Bashan A, Agmon I, Zarivach R, Schluenzen F, Harms J, Berisio R, Bartels H, Franceschi F, Auerbach T, Hansen HAS, 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

Belousoff MJ, Tjioe L, Graham B, Spiccia L (2008) Synthesis, X-ray crystal structures, and phosphate ester cleavage properties of bis(2-pyridylmethyl)amine

copper(II) complexes with guanidinium pendant groups. Inorg Chem 47 (19):8641–8651

Bokov K, Steinberg SV (2009) A hierarchial model for evolution of 23S ribosomal RNA. Nature 457:977–980

Gluehmann M, Zarivach R, Bashan A, Harms J, Schluenzen F, Bartels H, Agmon I, Rosenblum G, Pioletti M, Auerbach T, Avila H, Hansen HA, Franceschi F, Yonath A (2001) Ribosomal crystallography: from poorly diffracting micro-crystals to high resolution structures. Methods 25:292–302

Harms JM, Wilson DN, Schluenzen F, Connell SR, Stachelhaus T, Zaborowska Z, Spahn CM, Fucini P (2008) Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin. Mol Cell 30:26–38

Hornshaw MP, McDermott JR, Candy JM, Lakey JH (1995) Copper binding to the N-therminal tandem repeat region of mammalian and avian prion protein: structural studies using synthetic peptides. Biochem Biophys Res Comm 214 (3):993–999

Kim EE, Wyckoff HW (1991) Reaction mechanism of alkaline phosphatase based on crystal structures: two-metal ion catalysis. J Mol Biol 218(2):449–464

Kun A, Pongor S, Jordan F, Szathmary E (2007) Catalytic propensity of amino acids and the origins of the genetic code and proteins. In: Barbieri M (ed) The codes of life: the rules of macroevolution, 1st edn. Springer

Nissen P, Ippolito JA, Ban N, Moore PB, Steitz TA (2001) RNA tertiary interactions in the large ribosomal subunit: the A-minor motif. Proc Natl Acad Sci USA 98(9): 4899–4903

Okhapkin IM, Bronstein LM, Makvaeva EE, Matveeva VG, Sulman EM, Sulman MG, Khokhlov AR (2004) Thermosensitive imidazole-containing polymers as catalysts in hydrolytic decomposition of p-nitrophenyl acetate. Macromolecules 37:7879–7883

Roth A, Breaker RR (1998) An amino acid as a cofactor for a catalytic polynucleotide. Proc Natl Acad Sci USA 93:4537–4542

Sannes-Lowery KA, Hu P, Mack DP, Mei H-Y, Loo JA (1997) HIV-1 Tat peptide binding to TAR RNA by electrospray ionization mass spectrometry. Anal Chem 69 (24):5130–5135

Sobolevsky Y, Frenkel ZM, Trifonov EN (2007) Combinations of ancestral modules in proteins. J Mol Evol 65:640–650

Syme CD, Nadal RC, Rigby SEJ, Viles JH (2004) Copper binding to the Amyloid-β (Aβ) peptide associated with Alzheimer's Disease. J Biol Chem 279(18):18169–18177

Szathmary E (1993) Coding coenzyme handles: a hypothesis for the origin of the genetic code. Proc Natl Acad Sci USA 90:9916–9920

Trifonov EN (2009) The origin of the genetic code and of the earliest oligopeptides. Res Microbiol. doi:10.1016/j.resmic.2009.05.004

Trifonov EN, Frenkel ZM (2009) Evolution of protein modularity. Curr Opin Struct Biol 2009 19:335–340

Wulff G (2002) Enzyme-like catalysis by molecularly imprinted polymers. Chem Rev 102 (1):1–25

Young MJ, Wahnon D, Hynes RC, Chin J (1995) Reactivity of copper(II) hydroxides and copper(II) alkoxides for cleaving an activated phosphate diester. J Am Chem Soc 117:9441–9447

E-mail: ada.yonath@weizmann.ac.il