ORIGIN OF GENETICALLY ENCODED PROTEIN SYNTHESIS: A MODEL BASED ON SELECTION FOR RNA PEPTIDATION

J. TZE-FEI WONG

Department of Biochemistry, Hong Kong University of Science & Technology, Clear Water Bay, Hong Kong

(Received September 17, 1990)

Abstract. The difficulty in explaining the origin of genetic coding centres on the need to identify selective advantages that could account for the synthesis of peptidyl-tRNA, the essential intermediate in genetically programmed translation. It is resolved by a recognition of the functional advantages derivable from the post-transcriptional addition of peptide cofactors to RNA apo-catalysts. This enables the formulation of a theory for the origin of the genetic encoding of protein synthesis by RNA.

Introduction

The genetic code assigns 64 triplet RNA codons to 20 amino acids and termination signals. It enables the translation of genetic information stored in nucleic acids into proteins. For years the relative primacy of proteins and nucleic acids in the origin of life has posed a dilemma. More recently the discovery of catalytic RNA has suggested that the embodying of both coding and catalytic functions in the same RNA macromolecules might enable these molecules to evolve prior to the development of the genetic code and proteins [1–3]. Since the amino acid side chains of proteins are much more proficient in catalysis than the structural components of RNA, the RNA coding of proteins not surprisingly has led to the superseding of RNA catalysts by proteins. However, the puzzling question is, under the circumstances of the RNA world, what could be the nature of the selective steps that gave rise to genetic coding, and in so doing established the cooperation between nucleates and polypeptides that is fundamental to life today?

Evolution acts only on the present, and not on the anticipated future. Accordingly, genetic coding could not emerge based on some distant promise held out by an RNA-encoded, protein-catalyst world that had yet to be constructed. Instead, it had to be accomplished through an unbroken chain of steps, each attended by an immediate selective advantage. Therefore it becomes necessary to enquire into the kind of immediately advantageous steps that, without aiming at genetic coding, nonetheless arrived ultimately at genetic coding. Previously, Orgel [4] proposed that attachment of an amino acid or a dipeptide to the 3′-hydroxyl group of RNA could help mark the site for initiation of transcription. This would provide an incentive to the synthesis of aminoacyl-tRNA and dipeptidyl-tRNA, but not RNA compounds containing longer peptides, or peptides of defined sequences. Indeed, the lability of the O-aminoacyl ester bond is such that the 3′-hydroxyls might not even be the initial sites of useful attachment of amino acids and peptides to RNA.

Origins of Life and Evolution of the Biosphere **21**: 165–176, 1991.

Therefore the object of this study is to examine the nature of catalytic and other functional advantages of RNA peptidation that could lead to the evolution of elongated peptides of defined sequences on RNA and bring about the modern process of genetic coding.

Predisposing Factors

It has been recognized that some of the features of the RNA world might furnish important predisposing factors toward the development of genetic coding. Some bacterial and plant RNA viruses have tRNA-like structures at their 3′ ends which might function as an origin of replication and as a telomere. Accordingly tRNA-like structures could provide genomic tags in the RNA world to ensure completeness of RNA replication [5]. Moreover, many enzymes that utilise a nucleotide cofactor contain nucleotide-binding domains that could be derived from RNA-binding domains from precellular times [6]. Specific interactions between RNA and amino acids also have been observed [7, 8]. Bonding of chemical groups including amino acids to a polyanion such as RNA also could help to concentrate these groups on a cationic surface, and promote their participation in abiotic reactions [9].

The preexistence of tRNA-like structures and the specific interactions of RNA with amino acids and polypeptides all would favour a cooperation between the RNA and amino acid-polypeptide systems, and predispose the precellular formation of a genetic code. However, they fall short of defining a plausible chain of selective steps that would give rise to peptidyl-RNAs with defined sequences, and hence RNA encoded translation.

Post-Translational Modifications

One way to identify the factors within the RNA world that could confer selective advantages on the evolving RNA catalysts is to examine the factors that have been advantageous to present-day protein catalysts. In this regard, the most sustained source of improvement of protein function is evidently that furnished by an increased variety of amino acid side chains. Analysis of the structure of the genetic code has led to the coevolution theory that the early genetic code coevolved with amino acid biosynthesis. Primitive amino acids that were produced by prebiotic reactions gave rise to novel amino acids, and transferred part or all of their triplet codons to the latter. This accounts for the strong correlation between codon allocation and biosynthetic relationships among the amino acids, as well as the lack of prebiotic-type synthesis for many of the amino acids used in proteins to-day [10–14]. Even after the mainline genetic code was established, numerous amino acid side chains were added to proteins by post-translational modifications. There are more than 100 such additional side chains [15]. These developments attest to the powerful selective advantages constantly to be gained from having more amino acid side chains in the proteins.

Some of the post-translational modifications confer on the proteins a covalently-linked prosthetic group such as flavin or retinene, which fulfills a function that is beyond the capabilities of the 20 encoded amino acids. Moreover, in the addition of for example a myristoyl group, which helps to anchor proteins to lipid membranes [16], or a heme group, which enables the proteins to undergo redox reactions, post-translational modification is in fact far more appropriate than direct genetic encoding for the purpose of introducing such groups into proteins. Amino acids derivatized with a myristoyl or heme side chain would be so insoluble in the aqueous phase as to preclude their bonding to tRNA at a significant rate. Similarly, ribonucleotide reductase contains an essential free radical generated by the modification of a Tyr side chain [17]. Since the free radical is unstable and must depend on protein structure for stabilisation, only post-translational modification and not direct genetic encoding could be effective for its introduction into the protein.

RNA Peptidation

Given the wide-ranging, fundamental advantages gained by protein catalysts from post-translational modifications, ribozymes evolving in the RNA world could hardly be expected to forgo the vast benefits derivable from post-transcriptional modifications. Indeed, since it would be easier to introduce an additional amino acid into the genetic code than to introduce an additional base into RNA, on account of the structural constraints imposed by complementary base-pairing during transcription, post-transcriptional modifications would be even more important to catalytic RNAs than post-translational modifications are to proteins. Even to-day, many post-transcriptional modifications of RNA are known for messenger, transfer, ribosomal as well as viral RNA (Table I). Although the formations of O-aminoacyl-tRNA and O-peptidyl-tRNA are considered nowadays to be part of the pathway for protein biosynthesis, such reactions occurring prior to the development of protein biosynthesis would represent a particular class of post-transcriptional modifications, just like O- or N-methylation.

TABLE I

Occurrence of Post-transcriptional Modefications of RNA

Modification	Occurrence	Product
O-Methylation	tRNA, rRNA	O-methyl nucleosides
Base modifications (non-methylation)	tRNA, rRNA	Thio-U, pseudo-U, inosine, wyosine, m^7G, etc.
5'-Capping	mRNA, vRNA	$m^7G(5')ppp(5')Np....$
3'-Polyadenylation	mRNA	Addition of poly A tail
Splicing and editing	precursor RNA,	Mature mRNA, rRNA, tRNA
N-aminocylation	tRNA	Thr- and Lys-containing nucelosides
O-Aminoacylation and peptidation	tRNA	Intermediates of protein biosynthesis

Among the various RNA modifications, the addition of a lysine moiety to cytidine to form lysidine [18], the N-modified adenosines that contain a threonine, and a peptide-like structure involving threonine [19] are of particular interest, for they exemplify the useful post-transcriptional additions of amino acid and peptide-like moieties to RNA. In fact, N-aminoacyl and N-peptidyl compounds would be chemically more stable than their O-aminoacyl and O-peptidyl counterparts containing labile ester linkages.

Thermally or with condensing agents, oligopeptides could be formed from amino acids under prebiotic conditions [20, 21]. Thus both amino acids and peptides would be available in the primordial surroundings for reaction with RNA. Reactions that formed aminoacyl-RNA, for instance by a splicing-type reaction [5], could also give rise to peptidyl-RNA. Among contemporary organisms, compounds containing peptide-like moieties are not confined to RNA, but extend to a variety of nucleotides (Table II). The existence of this diverse range of compounds suggests the occurrence of widespread peptidations of nucleotides early in evolution. Since RNAs contain much the same chemical groups as free nucleotides, there is no cause for them to be exempt from peptidations.

Under prebiotic conditions, the addition of even an amino acid or a small peptide to the RNA molecules can expand significantly the range of their chemical and catalytic reactions. In Table II, glycyl-$tRNA_1$, folic acid, Factor 420 and Carbon Dioxide Reduction Factor are all catalytic cofactors. Amino acid side chains are adapt at catalysis, and could act in concert with the intrinsic catalytic groups on RNA. Nowadays, the outstanding performance of proteins as catalysts depends on a proper juxtaposition of catalytic amino acid groups. Much of the protein

TABLE II

Peptide-containing Nucleotidyl and Related Compounds

Compound	Nature of base-peptide link	Function
Glycyl-$tRNA_1$	O-peptidylnucleoside	Bacterial cell wall synthesis
UDP-N-acetylmuramyl-pentapeptide	UDP-GNAc-Lactyl-peptide	Bacterial cell wall synthesis
Coenzyme A	ADP-pantothenyl-cysteamine	Acyl transfer
Folic acid	Pteroyl-poly-glutamate	One-carbon transfer
Factor 420	Flavinoid-linked $(Glu)_2$	One-carbon transfer [22]
Carbon Dioxide Reduction Factor	Furanoid-linked $(Glu)_2$	One-carbon transfer [22]
Actinomycin	Phenoxazone-di-cyclo-pentapeptide	Antibiotic
Adenylated protein	Ribose-phospho-tyrosine	Protein regulation
ADP-ribosylated protein	N- and C-glycosides	Protein regulation
Viral RNA-protein	5'-Protein-pUUAAAACAG- for polio virus	Primer in RNA synthesis [23]
N-peptidyl-tRNA	purine-6-carbamoyl-threonyl-amido group	Transfer RNA [19]
O-peptidyl-tRNA	O-peptidyl-nucleoside	Intermediate in protein synthesis

molecule plays the role of a scaffold that optimally positions the catalytic groups, which explains the difficulty of obtaining model enzymes consisting of small peptides, since these usually do not incorporate a stable scaffold [24]. By designing an α-helical scaffold of as few as 10 residues to support catalytic groups, it has been possible to obtain a peptide with glycosidase activity [25]. A more complex scaffold also enables a 34-residue polypeptide to display ribonuclease activity [26]. In this regard, RNA could indeed provide a useful scaffold to position catalytic amino acid side chains. The secondary and tertiary structures of RNA are often more hardy than those of proteins. Transfer RNA can be reversibly melted by high temperatures without loss of function. In contrast, not many proteins can undergo reversible thermal denaturation. In prebiotic times, prior to the rise of oxygen and an oxidizing atmosphere, disulfide bonds would not form readily. The lack of protein stabilisation by disulfides would further favour RNA relative to proteins as primitive scaffolds.

Furthermore, the strongly anionic RNA suffers from the deficiency that it does not interact well with hydrophobic membranes, important sites for the development of bioenergetic processes. Attachment of a hydrophobic amino acid or better still a small hydrophobic peptide to the RNA would overcome this deficiency and allow an all-important coupling between replication and bioenergetics. For example, the peptides gramacidin A (15 amino acids) and valinomycin (6 amino acids alternating with 6 hydroxyamino acids in a cyclized structure) even facilitate the transport of potassium ions through membranes. Also, the biosynthesis of secretory proteins is accompanied by their co-translational insertion through the endoplasmic reticulum membrane guided by a signal peptide sequence on the protein. The peptidyl moiety remains covalently attached to the tRNA during the insertion. This behaviour demonstrates graphically how RNA-membrane association may be achieved through the attachment of an appropriate peptide to the RNA.

Origin of Genetically Encoded Protein Synthesis

Based on the benefits of post-transcriptional modifications, the wide occurrence of compounds containing both a peptide-like structure and a nucleotide or related nitrogenous base, and the known RNA modifications incorporating threonine, N-substituted threonine amide and lysine moieties, there is sufficient ground to propose that aminoacylation and peptidation of RNA likely played a major role in precellular metabolism. This proposal provides a model mechanism for the origin of genetic coding within the RNA world:

Stage 1

RNAs and oligopeptides coexisted in the precellular milieu. The evolving RNAs benefited from the inventive evolution [12] of post-transcriptional modifications. The covalent structures introduced into RNA components included N- and O-modifications containing amino acids and peptides, as well as linkages to the phosphate group to yield aminoacyl-AMP and peptidyl-AMP type of compounds.

These modifications were accomplished by various chemical mechanisms, not the least being crosslinking brought about by ultraviolet radiation, which was plentiful in the prebiotic environment prior to the rise of oxygen and hence the ozone shield. At first, modifications more stable than the relatively labile O-esters would play a more prominent role.

STAGE 2

Aminoacyl and peptidyl RNAs by virtue of their amino acid side chains represented functionally a particularly important class of modified RNAs. Their synthesis, initially from a direct bonding of preexistent amino acids and oligopeptides to the RNA, brought substantial evolutionary advantages for they extended the catalytic range of the ribozymes much as an attached heme or flavin would extend the catalytic range of enzymes. While non-covalent peptide-RNA complexes were useful in this regard, covalent conjugates came to be preferred in time on account of their stability. Specificity in the selection of an amino acid or peptide for bonding to an RNA relied on various physicochemical factors. The specific interactions between $tRNA^{Phe}$ and aromatic amino acids [7], or between Arg and the Tetrahymena self-splicing ribosomal intron [8], point to the feasibility of stereochemical selectivity. In the genetic code, the hydrophobicity of anticodon-doublet on tRNA is correlated to that of its cognate amino acid [27]. This could be due to a direct interaction between the anticodon on a tRNA and its cognate amino acid, especially if the structure of primitive tRNA places the amino acid close to the anticodon [28]. Alternatively, it could result from the adsorption of both tRNA and amino acid to a catalytic surface with the appropriate characteristics. Thus hydrophilic anticodons and amino acids would adsorb to hydrophilic sites, while hydrophobic anticodons and amino acids would adsorb to hydrophobic sites [14]. In any event, peptidyl RNAs with an appropriate peptide sequence are functionally even more versatile than aminoacyl RNAs. As preformed oligopeptides became depleted from the prebiotic environment, these peptidyl RNAs had to be constructed from the still abundant amino acids. Amino acids could be added sequentially to an RNA by successive rounds of reaction of the RNA with an activated amino acid such as aminoacyl-AMP. As in the biosynthesis of gramacidin S or tyrocidine through successive rounds of reaction with aminoacyl-AMP [29, 30], specification of peptide sequence would depend on the ordered action of different catalysts. There was intense natural selection for peptide sequences that promoted essential functions such as those of RNA replicase, transpeptidase, and aminoacyl-RNA synthetases. Enhanced performance in such functions conferred an immense competitive edge to the evolving ribozymes.

STAGE 3

An efficient pathway to generate longer and more versatile peptides with an ordered sequence was to have a peptidyl RNA react with another aminoacyl or peptidyl RNA. The sequence in which different carrier or transfer RNAs contribute to a

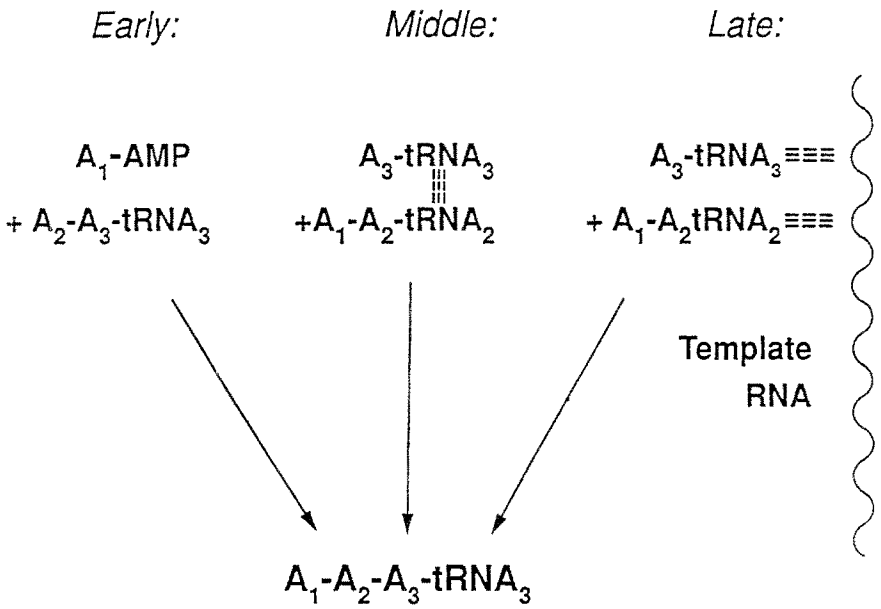

Fig. 1. Early, middle and late phases in the evolution of the mechanism for the synthesis of a tripeptide-tRNA.

growing peptide could be guided by base-pairing. While the stem-loop structure of tRNAs usefully reduces unintentional base-pairing [31], tRNAs with complementary anticodons are known to base-pair with high association constants that are modulated by post-transcriptional modifications [32]. Codon-induced interaction between tRNAs also has been observed [33]. Initially, different tRNAs might directly base-pair between themselves. For example, an ability of a Leu-Glu-tRNA to pair with an Asp-tRNA would facilitate the synthesis of Leu-Glu-Asp-tRNA or Asp-Leu-Glu-tRNA through transpeptidation. However, peptide sorting on this basis, even with the development of an N-terminus to C-terminus directionality that allowed the formation of Leu-Glu-Asp-tRNA but not Asp-Leu-Glu-tRNA in this instance, was too limited in scope. Therefore it was displaced in time by template-directed ordering, where different tRNAs were lined up for reaction through complementary codon-anticodon pairing to a template RNA. Figure 1 illustrates this evolution of peptidation mechanism for the elongation of a dipeptide-tRNA by one residue. Starting from an Early Phase reaction of the dipeptide-tRNA with aminoacyl-AMP, the mechanism progressed through a Middle Phase reaction between derivatized tRNAs aligned by direct tRNA-tRNA pairing, to finally a Late Phase tRNA-tRNA interaction mediated by side by side codon-anticodon pairing to template RNA.

Stage 4

The number of base pairs formed in the association between codon on template and anticodon on tRNA at first varied among competing systems at a comparable stage of development. Doublets suffered from the drawback of weak interaction,

while quadruplets or quintuplets were too cumbersome. The triplet system eventually proved to be optimal. O-peptidyl RNA, less favoured as post-transcriptional modifications earlier because of their instabilities relative to N-peptidyl and other modifications, gained prominence as the velocity of peptide growth became important. Acyl esters are more reactive than amides, and undergo more facile transpeptidations. As the peptidyl cofactors on the ribozymes grew longer, they managed to function as catalysts with less and less assistance from catalytic groups on the apo-ribozymes. Ultimately they became detached from the RNA. In so doing the peptidated RNAs were transformed from being holo-ribozymes to being mere intermediates in polypeptide synthesis. The optional detachment of a cofactor from a macromolecule is well known in the case of proteins. Heme is covalently attached to cytochrome c, but detached from cytochrome b or hemoglobin. Likewise flavin coenzymes are covalently bonded to some apoflavoenzymes but not others.

Stage 5

As the triplet code was established, novel amino acids were formed from precursor amino acids that were available prebiotically, and gained entry into the genetic code. This coevolution of the code and amino acid biosynthesis resulted in the genetic code of to-day for 20 universally encoded amino acids [10]. The code ceased to expand only when the chemical versatility of the amino acid ensemble permitted low-noise translation [11]. Thereafter later-arriving amino acid side chains gained entry into proteins only through post-translational modifications.

The plausible time-scenario incorporating Stages 1–5 of this RNA peptidation model for the emergence of genetic coding is summarized in Figure 2. The model depends for its construction on the benefits of post-transcriptional modifications, the formation of peptidyl-RNA, and the catalytic propensity of amino acid side chains.

2.5-3.0 × 10⁹ yr ago	Establishment of near-universal genetic code for 20 amino acids through coevolution of the code with amino acid biosynthesis.
	Triplet codon-anticodon pairing was selected over doublet, quadruplet, quintuplet pairing. The polypeptide cofactors functioned detached from ribozymes.
	Ordering of peptides sequence by base-pairing between tRNAs gave way to ordering by base-pairing between tRNAs and RNA template.
	Depletion of preexistant peptides led to synthesis of peptidyl RNA from aminoacyl RNA.
	Peptidyl-RNA proved to be the most advantageous form of post-transcriptionally modified RNA.
3.5-4.0 × 10⁹ yr ago	Starting point: prebiotic world of evolving RNAs and peptides.

Fig. 2. RNA-peptidation model for the evolutionary development of genetically encoded protein synthesis.

These are all established elements in present day metabolism. Because peptidyl-RNA is an essential intermediate in genetically encoded translation, the development of translation inevitably requires a selective advantage to account for the synthesis of this key intermediate. Full-fledged proteins, as yet unevolved in the RNA world, could not provide a starting point for retrograde evolution of peptidyl-RNA. In contrast, RNA peptidation, by improving the function of ribozymes, provided an immediate evolutionary incentive for the RNA world to develop peptidated RNAs. Moreover, because the catalytic activities of peptides vary with their amino acid sequences, the incentive called for defined sequences in the peptides to be attached to the RNA. With these peptidyl RNA intermediates of defined sequences providing the evolutionary impetus for their own synthesis, the difficulty of identifying a selective mechanism to account for their formation, leading the RNA world to develop into the RNA-protein world, is thus resolved.

Predictions of the Model

The RNA-peptidation model requires that the covalent addition of aminoacyl and peptidyl groups improved the performance of the RNA molecules either in catalysis or in other attributes such as their ability to interact with membranes. On this basis, it explains the transformation of the RNA world first of all into a peptidyl-RNA world, and eventually into an RNA-protein world. Subsequent emergence of DNA as a more stable storage of genetic information than RNA finally gave shape to the present-day DNA-RNA-protein world. The model makes a number of well defined predictions that are open to experimental testing:

(I) To verify the usefulness of peptidation to the ribozymes of the RNA world, it should prove possible to design and synthesize peptidated RNAs with enhanced capabilities compared to naked RNAs for catalysis or interaction with membranes and other chemical structures.

(II) The polypeptidyl-tRNA precursor for an enzyme should at least in some instances display part of the activity of the mature enzyme, thus establishing the capacity of peptidated RNAs to perform as catalysts.

(III) RNA catalysts, or plausible components of the RNA world such as clay surfaces, should be capable of catalysing the ligation of amino acids and peptides to RNA to make possible the synthesis of peptidated RNA.

(IV) RNA or peptidated-RNA catalysts should be capable of catalysing the formation of peptide bonds.

In ribosomal protein synthesis, since the presence of the tRNA moiety cannot prevent entirely the polypeptidyl-tRNA intermediates nearing chain completion to enter into the folding process, these intermediates are expected to take on some of the characteristics of the mature protein. Accordingly it should be possible to isolate such intermediates from different systems and determine their ability to perform some of the functions of the mature protein, thus testing the validity of Prediction II.

When RNA catalysis was first discovered, the catalysed reactions were restricted to the hydrolysis and transfer of the phosphodiester bonds of RNA itself. This limitation has since been relaxed [34]. There is at present no ground to preclude the catalysed reactions required by Predictions III and IV, even though their feasibility yet remains to be demonstrated. During protein biosynthesis, there is in fact extensive evidence in support of a requirement for rRNA in the chemical steps of the translation process [35].

With regard to Prediction I, it should be possible to attach hydrophobic amino acids or peptides to an RNA and improve its adsorption to biological membranes. Besides, the ribozymes so far observed are fairly sluggish catalysts with small catalytic rate constants. The cleavage of different hammerhead RNAs, for instance, displays half lives of 0.5 min or longer [36]. This in the face of the chemical instability of RNA limited severely the evolution of RNA catalysts. Not surprisingly, therefore, biological RNA catalysts mostly function nowadays only in intramolecular self-scission, as in the case of the Tetrahymena LSU intron, or in association with a protein factor that enhances the catalytic activity, as in the case of RNase P where the protein component increases the V_m by 20-fold [37]. It will be of great interest to determine if the attachment of amino acids and peptides could enhance the catalytic performance of ribozymes sufficiently to overcome the weakness of their tardiness, which is undoubtedly the Achilles' heel of the RNA world. A rate enhancement by one to two orders of magnitude might be needed.

In this regard, because the secondary structure of RNA and DNA is highly regular in the double-stranded regions, it should be possible to design and synthesize a double-stranded RNA or DNA segment having specified base residues prederivatized with an amino acid or peptide. This is exemplified by a double-helical fragment such as

C-C-G-C-G-X-C
G-Y-C-G-C-G-G

where *X* is an amino acid or peptide-modified *C*, and *Y* is an amino acid or peptide-modified *G*. The regular spacing of the bases along the double-helix will define narrowly the spacing between the amino acid sidechains on *X* and *Y*. By introducing the sidechains of Asp, Ser, His as well as other amino acids into the double-helix esterase and other enzymic activities may be sought. The distance between *X* and *Y* may be varied, and two or more modified bases may be placed on the same or opposite strands of the double helix. Of course, more elaborate catalytic configurations that utilise more complex tertiary foldings of RNA and DNA, and the functional groups on both the amino acids and the nucleotides themselves, also may be devised.

If the RNA-peptidation model finds support in the observation of enhanced catalytic activities of amino acid- and peptide-derivatized nucleic acids, it would point to the original synthesis of polypeptides as covalently bound cofactors for the self-replicating ribozymes. Eventually they became so successful as catalysts

themselves as they grew longer and folded into magnificent structures that the RNAs largely relinquished their catalytic activities, retaining along with their DNA partners only the replicative and encoding roles. The polypeptides acquired the capacity to function detached from their apo-ribozymes, and become known as enzymes. In this light, the RNA world did not develop genetic coding and RNA-programmed translation in order to make proteins. Instead, the biosynthesis of proteins was merely the ultimate, accidental extension of the prolonged evolution of peptidated RNA.

Acknowledgements

I am indebted to Drs. B. G. Lane and S. L. Miller for valuable discussion and the Medical Research Council of Canada for support.

References

[1] Cech, T. R. and Bass, B.: 1986, *Annu. Rev. Biochem.* **55**, 599–629.
[2] Darnell, J. E. and Doolittle, W. F.: 1986, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1271–1275.
[3] Gilbert, W.: 1986, *Nature* **319**, 618.
[4] Orgel, L. E.: 1989, *J. Mol. Evol.* **29**, 465–474.
[5] Weiner, A. M. and Maizels, N.: 1987, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7383–7387.
[6] White III, H. B.: 1976, *J. Mol. Evol.* **7**, 101–104.
[7] Bujalowski, W. and Porschke, D.: 1988, *Biophys. Chem.* **30**, 151–157.
[8] Yarus, M.: 1988, *Science* **240**, 1751–1758.
[9] Wachtershauser, G.: 1988, *Microbiol. Rev.* **52**, 452–484.
[10] Wong, J. T.: 1975, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1909–1912.
[11] Wong, J. T.: 1976, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2336–2340.
[12] Wong, J. T.: 1981, *Trends Biochem. Sci.* **6**, 33–35.
[13] Wong, J. T.: 1984, in Ricard, J. and Cornish-Bowden, A. (eds.), *Dynamics of Biochemical Systems*, Plenum Press, New York pp. 247–258.
[14] Wong, J. T.: 1988, *Microbiol. Sci.* **5**, 174–181.
[15] Uy, R. and Wold, F.: 1977, *Science* **198**, 890–896.
[16] Towler, D. A. and Gordon, J. I.: 1988, *Annu. Rev. Biochem.* **57**, 69–99.
[17] Reichard, P. and Ehrenberg, A.: 1983, *Science* **221**, 514–519.
[18] Muramatsu, T., Nishikawa, K., Nemoto, F., Kuchion, Y., Nishimura, S., Miyazawa, T., and Yokoyama, S.: 1988, *Nature* **336**, 179–181.
[19] Nishimura, S.: 1979, in Schimmel, P. R., Soll, D., and Abelson, J. N. (eds.), *Transfer RNA: Structure, Properties and Recognition*, Cold Spring Harbour Laboratory, pp. 547–549.
[20] Rohlfing, D. L.: 1976, *Science* **193**, 68–70.
[21] Kenyon, D. H. and Steinman: 1969, *Biochemical Predestination*, Mcgraw-Hill New York, pp. 182–195.
[22] Walsh, C. T.: 1984, *Trends Biochem. Sci.* **9**, 159–162.
[23] Nomoto, A., Detjen, B., Pozzatti, R., and Wimmer, E.: 1977, *Nature* **268**, 208–213.
[24] Robson, B.: 1980, *Trends in Biochem. Sci.* **5**, 240–244.
[25] Chakravarty, P. K., Mathur, K. B., and Dhar, M. M.: *Experientia* **229**, 786–788.
[26] Gutte, B., Daumigen, M., and Wittschieber, E.: 1979, *Nature* **281**, 650–655.
[27] Lacy, C. L. and Mullins: 1983, *Origins of Lige* **13**, 3–42.
[28] Hopfield, J. J.: 1978, *Proc. Natl. Acad. Sci.* **75**, 4334–4338.
[29] Lee, S. G., Roskoski Jr., Bauer, and Lipmann, F.: 1973, *Biochemistry* **12**, 398–405.
[30] Lipmann, F.: 1974, in Dose, K., Fox, S. W., Deborin, G. A., and Pavlovskaya, T. E. (eds.), *Origin of Life and Evolutionary Biochemistry*, Plenum Press, New York and London, pp. 321–330.

[31] Wang, Q. S., Bronskill, P. M., and Wong, J. T.: 1987, *Arch. Biochem. Biophys.* **255**, 176–183.
[32] Weissenbach, J. and Grosjean, H.: 1981, *Eur. J. Biochem.* **116**, 207–213.
[33] Labuda, D. and Porschke, D.: 1983, *J. Mol. Biol.* **167**, 205–209.
[34] Shvedova, T. A., Korneeva, G. A., Otroshchenko, V. A., and Venkstern, T. V.: 1987, *Nucleic Acids Res.* **15**, 1745–1752.
[35] Noller, H. F., Stern, S., Moazed, D., Powers, T., Svensson, P., and Changchien, L. M.: 1987, *Cold Spring Harbor Symp. Quant. Biol.* **52**, 695–708.
[36] Ruffner, D. E., Dahm, S. C., and Uhlenbeck, O. C.: 1989, in Belfort, M. and Shub, D. A. (eds.), *RNA: Catalysis, Splicing, Evolution*, Elsevier Amsterdam, pp. 31–41.
[37] Pace, N. R., Reich, C., James, B. D., Olsen, G. J., Pace, B., and Waugh, D. S.: 1987, *Cold Spring Harbor Symp. Quant. Biol.* **52**, 239–248.