A HYPOTHESIS: RECIPROCAL INFORMATION TRANSFER BETWEEN OLIGORIBONUCLEOTIDES AND OLIGOPEPTIDES IN PREBIOTIC MOLECULAR EVOLUTION *

SHUGUANG ZHANG
Department of Biology 68-233, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

and

MARTIN EGLI
Organic Chemistry Laboratory CHN-H25, ETH-Swiss Federal Institute of Technology, Universität-Strasse 16, CH-8092, Zürich, Switzerland

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Abstract. We wish to propose a mechanism for reciprocal information transfer in prebiotic molecular evolution, based on heterologous pairing complex formation between oligoribonucleotides and oligopeptides. In this proposed pairing complex, the bases of the oligoribonucleotide and the side chains of the oligopeptide may form three types of complementary Watson-Crick-type hydrogen bonds. The structural basis for the pairing is the close correspondence of the distances between the side chains in the two molecules. Both the inter-nucleotide spacing of the RNA and the inter-side-chain spacing of the peptide are approximately 3.4 Å. The proposed pairing mode would allow both specific and nonspecific interactions required for reciprocal information transfer. Thus, it represents a simple and versatile coding system that could have had significant implications in prebiotic molecular selection and evolution. In addition, we propose several testable experimental approaches based on the pairing mode of oligoribonucleotides and oligopeptides to verify our hypothesis.

Introduction

Information transfer between nucleic acids has been firmly established since the discovery of the DNA double helix structure [1]. Information can flow between DNA molecules, as well as between DNA and RNA [1–7]. Transfer of information from RNA to protein via transfer-RNA (tRNA) has also been verified in all living systems and in cell-free systems [8-9]. Information transfer from DNA to RNA to protein constitutes the Central Dogma of Molecular Biology [10–11]. The Central Dogma states that once information has passed into protein it cannot get out again [10]. Accordingly, direct information exchange between nucleic acids and peptides without tRNA has remained elusive. Nevertheless, there still remains the fundamental question of how information could have flown between nucleic acids and peptides under prebiotic conditions in the origin of life when there was no sophisticated biological molecular machinery for information transfers in replication, transcription and translation.

We have recently proposed a structural model (E-Z model) for a heterologous pairing complex between a single-stranded nucleic acid and a β-stranded peptide

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[12]. Such a pairing between an oligoribonucleotide and an oligopeptide is based on the fact that the distances between the side chains attached to the backbones of the two molecules show close correspondence, approximately 3.4 Å in both cases. In other words, the distance is approximately 6.8 Å between side-chains pointing to the same side of the peptide backbone in a β-strand containing all L-amino acids [13]. Similarly, the distance between every other base along the nucleic acid phosphodiester backbone is also approximately 6.8 Å [1]. Thus every other base on the ribonucleotide could form a pairing with the side chains of a β-stranded peptide. In addition to this structural compatibility, several complementary pairings between the bases of nucleic acids and the side chains of peptides can be formed through two hydrogen bonds. Here, we describe a simple and versatile molecular coding system for reciprocal information transfer between nucleic acids and peptides that may have been relevant in prebiotic molecular evolution.

Chemical and Structural Basis of the Pairing and A Simulated Pairing Model

COMPLEMENTARY PAIRINGS BETWEEN NUCLEIC ACID BASES AND AMINO ACID SIDE CHAINS VIA TWO WATSON-CRICK-TYPE HYDROGEN BONDS

There are three Types of Watson-Crick-type hydrogen bond complementary pairings between nucleic acid bases and amino acid side chains [12 and references therein]. Each pairing mode allows the formation of at least two hydrogen bonds. The specific Type I pairing between guanine (G) and aspartic acid (Asp) or glutamic acid (Glu), and the specific Type II pairing between cytosine (C) and arginine (Arg) or lysine (Lys) are achieved through formation of two hydrogen bonds. In Type I and Type II pairings between a base and the side-chain of an amino acid, both hydrogen donors are located on one molecule and the two acceptors are located on the other. For example, in the Type I pairing, the hydrogen donors are located on guanine at positions 1 and 2, and the acceptors are located on the carboxylic groups of Asp and Glu; in the Type II pairing, the hydrogen donors are located on the guanidinium group of Arg and the ζ-amino group of Lys, and the acceptors are located on cytosine at positions 2 and 3. Type III pairings are nonspecific and can occur between adenine (A), uracil (U), guanine (G), cytosine (C), inosine (I) or xanthine (X) and asparagine (Asn) or glutamine (Gln). In these pairs, each base and amino acid act both as a hydrogen donor and a hydrogen acceptor. These specific and nonspecific pairs are listed in Table I (The detailed schematic pairing modes have been presented in Figure 1 of reference [12]).

A PAIRING MODE BETWEEN OLIGORIBONUCLEOTIDES AND OLIGOPEPTIDES

We recently proposed a structural model for a heterologous RNA-peptide pairing complex utilizing alternating repetitive sequences to demonstrate the geometrical complementarity and structural compatibility between nucleic acids and peptides [12]. However, alternating repeats are not a prerequisite for such a pairing com-

TABLE I

Specific and nonspecific Watson-Crick-type hydrogen bond pairings between nucleic acid bases and amino acid side-chains

(A) Specific hydrogen bonds			(B) Non-specific hydrogen bonds		
Type	Base	Amino acid	Type	Base	Amino acid
I	Guanine	Asp, Glu	III	Guanine	Asn, Gln
II	Cytosine	Arg, Lys	III	Cytosine	
			III	Adenine	
			III	Uracil	
			III	Inosine	
			III	Xanthine	

The specificity of pairings is based on the particular positions of hydrogen donors and acceptors in the components [34–36, summarized in 37]. In Type I pairing, guanine is the only base that possesses two hydrogen-donating groups next to each other (N1, N2), and can thus pair specifically with the carboxylic groups of Asp and Glu. Similarly, in Type II pairing, cytosine is the only base that has two hydrogen-accepting groups next to each other (O2, N3), and can thus pair specifically with the guanidinium group of Arg, as well as with the protonated amino nitrogen atoms of Lys. In Type III pairing, Asn and Gln can form at least two hydrogen bonds with not only all naturally occurring nucleic acid bases, but also with metabolic intermediates, such as inosine and xanthine. The detailed pairing interactions have been presented in a previous communication [12].

plex. Rather, a wide variety of complementary oligoribonucleotides and oligopeptides may form such pairing complexes. An example of such a pairing is shown in Figure 1. The sequence of the oligoribonucleotide is 5′-ACGUAUACGUGC-3′, and the sequence of the peptide is Asn-Gly-Asp-Ala-Asn-Ala-Asn-Ala-Asp-Ala-Asp-Gly. The RNA sequence is non-self-complementary and self-association is therefore avoided. Moreover, the chosen sequence does not allow staggered arrangements among oligoribonucleotides so as to avoid aggregation. It was suggested that under prebiotic conditions replication of sequences with alternating purines and pyrimidines may have had some advantages in comparison with those of non-alternating nucleotide sequences [14]. Hence this could have led to an accumulation of such oligonucleotides. Moreover, it has been shown that short peptides with alternating hydrophilic and hydrophobic residues favor β-sheet conformation in water, and that the simplest oligopeptides had a tendency to form β-sheets under the assumed prebiotic conditions [15–16]. Since alternating sequences will self-associate more easily, rapid degradation could have been avoided and this would have resulted in an accumulation of such peptides [15]. The amino acid composition chosen for the oligopeptide in the pairing model is consistent with the abundance of certain amino acids produced under assumingly prebiotic conditions.

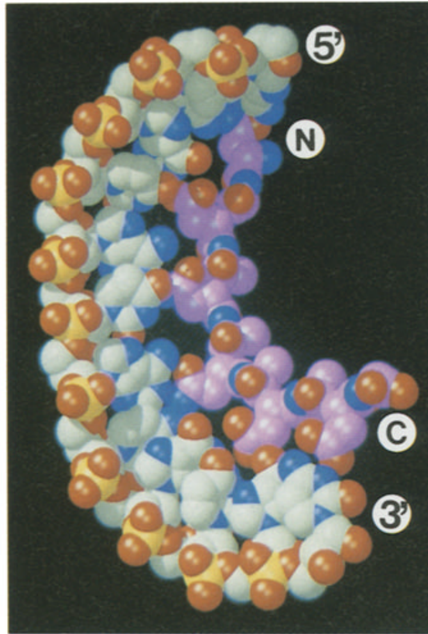


Fig. 1. Van der Waals model of a heterologous pairing complex between the dodeca-ribonucleotide 5′-ACGUAUACGUGC-3′ and the β-stranded dodeca-peptide Asn-Gly-Asp-Ala-Asn-Ala-Asn-Ala-Asp-Ala-Asp-Gly. The riboses of the oligonucleotide backbone adopt C_3'-*endo* pucker. Bases A and G form two hydrogen bonds with amino acids Asn and Asp, respectively. Bases U and C are not paired with any amino acids, rather they are stacked between purine bases. In addition Ala and Gly do not form any hydrogen bonds with bases but their hydrophobic interactions may facilitate formation of the heterologous pairing complex. The average helical rise of the pairing complex is about 3.4 Å. RNA carbon atoms are white and peptide carbon atoms are violet, phosphorus atoms are yellow, oxygen atoms are red, and nitrogen atoms are blue. Van der Waals radii were reduced slightly and hydrogen atoms were omitted for clarity. N and C refer to the N- and C-termini of the oligopeptide. The 5′ and 3′ refer to the 5′- and 3′- ends of the oligoribonucleotide.

In Miller-Urey type experiments, it was shown that the most abundant biologically relevant amino acids formed were Gly, Ala, Asp, Asn and Glu [17–18].

The heterologous pairing complex model depicted in Figure 1 was constructed in a similar way as described in detail previously [12]. To combine the two molecules, the RNA strand was unwound without altering the C3′-*endo* conformation of its riboses, and the ideal β-strand conformation of the peptide was given a slight right-handed twist, commonly observed with β-sheets [19–20]. In the pairing complex presented here the RNA and peptide backbone conformations are necessarily less homogeneous compared to a pairing complex consisting of an RNA and a peptide strand paired only via Type I and Type II pairings [12]. This is due to the relative shifts between hydrogen-bonded bases and amino acid side chains in pairs of Types I and II on the one hand, and in pairs of Type III on the other.

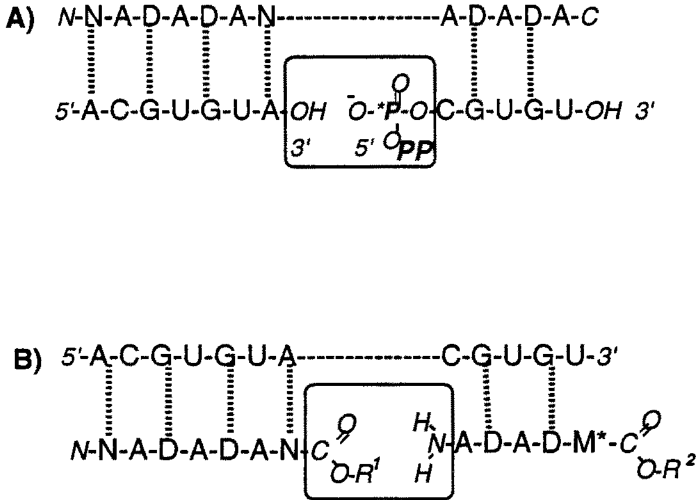


Fig. 2. Proposed experimental schemes. (A) An oligopeptide Asn-Ala-Asp-Ala-Asp-Ala-AsnAla-Asp-Ala-Asp-Ala serves as the template for ligation of two oligonucleotides 5′-^{32}*P*-ACGUGUA-OH-3′ and 5′-*PPP*-CGUGU-OH-3′. (B) An oligoribonucleotide 5′-ACGUGUACGUGU-3′ serves as the template for condensation of two oligopeptides *N*-Asn-Ala-Asp-Ala-Asp-Ala-Asn-*C* (NADADAN) and Ala-Asp-Ala-Asp-Met(^{35}*S*)(ADADM). The backbones are shown schematically and hydrogen bonding interactions are indicated by dashed lines. The oligopeptide *N*- and *C*- termini are labeled, and the termini of oligonucleotides are labeled 5′ and 3′. The possible reaction areas are boxed. R^1 and R^2 refer to two different chemical groups. R^1 could be a phenyl group or other derivatives. *PP*- refers to a pyrophosphate leaving group. * refers to radioisotope labeled materials, ^{32}P for the penta-oligoribonucleotide and ^{35}S for the Met on the penta-oligopeptide, respectively.

PROPOSED EXPERIMENTS

The proposed pairing of oligoribonucleotides and oligopeptides via Watson-Crick-type hydrogen bonds is based on the remarkable structural compatibility of the backbones and the chemical complementarity of the bases of nucleic acids and the side chains of peptides. Such a pairing mode might allow reciprocol information transfer, and both molecules could thus serve as templates and substrates in a transfer of information. For example, if a dodeca-ribonucleotide with the sequence 5′-ACGUGUACGUGU-3′ served as the template, a heptapeptide Asn-Ala-Asp-Ala-Asp-Ala-Asn with an activated *C*-terminus, such as a phenyl ester, and a pentapeptide Ala-Asp-Ala-Asp-Met(^{35}S) could act as substrates (Figure 2b). The rate of amide bond formation between the α-carboxylic group of the *C*-terminal Asn on the heptapeptide and the α-amino group on the *N*-terminal Ala of the pentapeptide could probably be accelerated in the presence of the RNA template. Formation of the dodoca-peptide product and the rate of its formation can be readily monitored and analyzed following the radioactivity of the labeled oligopeptide. Similarly, if the oligopeptide Asn-Ala-Asp-Ala-Asp-Ala-Asn-Ala-Asp-Ala-Asp-Ala served as the template, a heptaribonucleotide 5′-^{32}P-ACGUGUA-OH-3′ and a penta-ribonucleotide 5′-ppp-CGUGU-OH-3′ could act as substrates (Figure 2a).

The rate of phosphodiester bond formation between the 3′-hydroxyl group of the hepta-ribonucleotide and the 5′-phosphate group of the pentanucleotide could probably be accelerated. As in the case of the peptide condensation, because of the ^{32}P-labeled nucleotide, formation of the dodeca-ribonucleotide product can be readily monitored and analyzed. For the proposed experiments, accumulated dodecameric RNA can be readily analyzed with gel electrophoresis. Likewise, the Met residue at the *C*-terminus of the pentapeptide Ala-Asp-Ala-Asp-Met(^{35}S) can be labeled, and accumulated dodeca-peptide can be analyzed in a similar way. In both reactions the templates may bring the substrates into close proximity through formation of complementary hydrogen bond pairings. This mechanism somewhat resembles the action of enzymes. For instance, synthetases enable two separate substrates to be brought into close proximity so as to accelerate the rate of bond formation via an entropic contribution [21].

It is very likely that a vast number of combinations of oligoribonucleotides and oligopeptides will have to be systematically tested in order to find some optimal experimental conditions and to verify our hypothesis of peptide-nucleotide heterologous pairing complex formation and reciprocol information transfer. A few selected sequences which in principle fulfill the requirements are listed in Table II. Many of the similar sequences listed in Table II have been found in current releases of nucleic acid and protein sequence data banks (S. Zhang, unpublished observation). It is possible that certain sequences may be more favorable as templates than others, depending on experimental conditions. It is likely that a great number of experimental parameters, e.g. ionic concentrations, metal cofactors, temperature, pH range and duration of reaction, etc., must be systematically tested before an optimal condition may be found and a reasonable assessment can be made. It must be emphasized that a large variety of sequences and lengths should be tested, and that one should not limit oneself to the sequences and sizes listed in Table II. Similarly, a variety of prevailing prebiotic conditions could have generated different environments leading to molecular selection and evolution.

Discussion

In spite of certain requirements for the sequences of oligoribonucleotides and oligopeptides to form a heterologous pairing complex, i.e. non-self complementary nucleic acids and β-strand forming oligopeptides, there remains a vast number of sequences that bear an intrinsic potential for reciprocal information transfer between the two molecules.

Our model is likely to facilitate two kinds of information transfer, one with high transfer fidelity and consequent conservation of information, the other with error tolerance and hence diversification. Although our proposed information transfer relies on the formation of base-amino acid combinations paired through Watson-Crick-type hydrogen bonds in either case, Type I and Type II pairings are formed with higher tendency of specific recognition, hence more faithful information

TABLE II

Partial list of sequences of oligoribonucleotides and oligopeptides that may serve as templates for reciprocal information transfer

Type pairing	No.	NAME	Oligonucleotides 5′ ⟶ 3′	NAME	Oligopeptides N ⟶ C
	1.	GU12	GUGUGUGUGUGU	DA12	DADADADADADA
	2.	GA12	GAGAGAGAGAGA	DF12, DG12, DH12, DI12, DK12, DL12	
I	3.	GAG	GAGUGUGAGUGA	DM12, DN12, DQ12, DR12, DS12, DT12	
	4.	GUG	GUGAGUGUGAGU	DV12, DW12, DY12	
	5.	GCG	GCGUGAGAGCGU	EX12, X = any of the above amino acids, except D	
	6.	CA12	CACACACACACA	KA12	KAKAKAKAKAKA
	7.	CU12	CUCUCUCUCUCU	KA12, KD12, KE12, KF12, KG12, KH12	
II	8.	CAC	CACUCUCACUCA	KI12, KL12, KM12, KN12, KQ12, KS12	
	9.	CGC	CGCUCACACGCU	KT12, KV12, KW12, KY12	
	10.	CUC	CUC ACACUCGCA	RX12, X = any of the above amino acids, except K	
	11.	GUA	GUACACGUACAC	DANANVDLNING, DGNANHDVNANA	
	12.	AAA	AAACGUACGCAU	QAQAELQIEVQQ, NANADINLDANN	
III	13.	CGU	GCGUACAUGUAA	DGDANANYDANG, EGEVQAQYEVQA	
	14.	CAC	CACGCAUGCAUA	KVKVKAQLKIQL, LQVEAQLEIQLQ	
	15.	UAC	UACAUGUGCACA	QVRAQAQIRGRG, VQVQAEAEVQVQ	

Single letter codes for both ribonucleotides and amino acids are used. For nucleotides, A = adenine, G = guanine, U = Uracil, and C = cytosine, For amino acids, A = alanine, D = aspartic acid, E = glutamic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, N = asparagine, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, and Y = tyrosine. E, Q and R can substitute D, N and K, respectively, as long as one follows the rules of structural compatibility. Oligoribonucleotides 1–5 can pair with any of the oligopeptides listed under Type I pairings. Likewise, oligoribonucleotides 6–10 can pair with any of the oligopeptides listed under Type II pairings. On the other hand, each of the oligoribonucleotides listed under Type III pairings can only pair with the two selected oligopeptides in the same line, but with none of the others in the table. Many more sequences which could form this kind of pairs can be proposed readily. The given sequences represent only a very small fraction of a large number of possible molecules.

transfer and less chance for diversification may be expected. Type III pairings, on the other hand, are less specific and can thus introduce diversification through error tolerance. The introduction of diversification is a prerequisite for prebiotic molecular selection and evolution [22], and such diversification could be one of the principles that underlie the variations now observed.

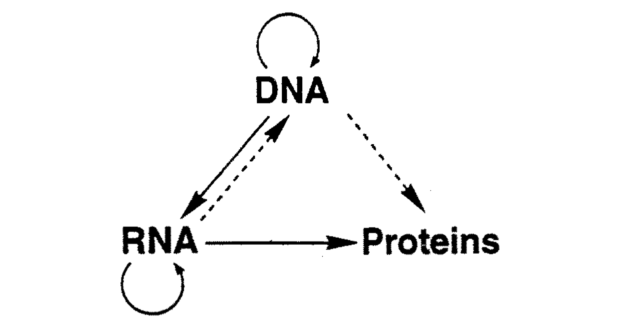
Advances in non-enzymatic peptide condensation and protein fragment re-formation in aqueous solution [23] suggest that specific template-mediated oligopeptide condensation be plausible. One of the examples of such a protein fragment re-formation was demonstrated by Corradin and Harbury [24]. They showed that

two separate cytochrome c fragments, 1-65 and 66–104, were spontaneously re-formed to reconstitute a biologically active cytochrone c [24]. Many examples of this kind of peptide condensations and spontaneous protein fragment re-formation including insulin, nucleases, and myoglobin, etc. have been reviewed [25]. Likewise, Bartel and Szostak have recently shown that an oligoribonucleotide and a hepta-oligoribonucleotide were able to self-ligate in the presence of a 13mer oligoribonucleotide as a template which brought the two separate oligoribonucleotides into close contact and correct orientation [26]. The end product is a ribonucleotide with a biologically relevant $3' \rightarrow 5'$ phosphodiester bond linkage [26–27]. The ligation reaction required magnesium, the 13mer oligoribonucleotide template and long duration of incubation [26]. Bartel and Szostak's results again set an example of information transfer between ribonucleic acids relevant in prebiotic evolution. This kind of reaction had been proposed by Rich more than thirty years ago concerning the origin of life [28].

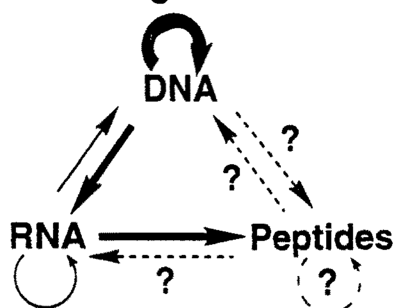
Our model of reciprocal information transfer between single-stranded oligoribonucleotides and β-stranded oligopeptides is also consistent with a proposal by Kauffman that nucleic acids and proteins in the prebiotic period could have co-evolved [29]. An experimental verification of our proposal could lead us one step closer to overcome the conceptual difficulty to imagine the reciprocal information transfer between nucleic acids and peptides in the origin of life and the origin of primitive chemical information code. Carter and Krant previously proposed a RNA/peptide interaction model in which two β-stranded peptides fit precisely into the minor groove of RNA [30]. Since the primary interactions in their model are through the backbones of the β-stranded peptides and the RNA riboses, the coding specificity for the reciprocal information transfer might be less effective. One of the important aspects of reciprocal information transfer in prebiotic environment is an easy separation of the copied strand. From the structural point of view, the formation of our proposed heterologous nucleic acid-peptide pairing complexes may be associated with relatively low melting point and may be less stable in comparison with nucleic acid homopolymers, hence separation of the heterologous oligomers might be readily achieved. When a complex of this kind of heterologous molecules reaches a less stable nevertheless self-organized critical state at the edge of chaos, random molecules may be driven toward a highly dynamic organization, therefore many impossible reactions could have been facilitated in such an environment.

Reciprocal information transfer as suggested by our structural model has the consequence that information flow may not be necessarily unidirectional, that is only from nucleic acid to protein, as stated by the Central Dogma of Molecular Biology (Figure 3a) [10–11]. Considering a prebiotic environment, it is not unreasonable to speculate that the copying mechanisms then were much simpler than the ones now encountered in biological systems. Information might have been directly exchanged between oligoribonucleotides, oligodeoxyribonucleotides and oligopeptides prebiotically in the origin of life. A recently described new class of self-complementary oligopeptides which may self-pair through ionic bond and

A) The Central Dogma of Molecular Biology



B) A Proposed Diagram in The Origin of Life



Net work Communication

Fig. 3. (A) The Central Dogma of Molecular Biology [10–11]. A proposed diagram in prebiotic molecular evolution. This hypothetical diagram illustrates all possible reciprocal information transfers in prebiotic molecular evolution. RNA, DNA, and proteins refer to oligoribonucleotides, oligodeoxyribonucleotides, and oligopeptides, respectively. Solid thick arrows indicate major information flows, i.e. DNA → DNA; DNA → RNA; and RNA → Protein [10–11]. Solid thin arrows indicate minor information flows, i.e. RNA → RNA [3] and RNA → DNA [4–5]. These directional information flows are encountered in biological systems. Dashed thin arrows suggest the reciprocal information flows during an early stage of prebiotic molecular evolution. A similar diagram was proposed by Crick [11].

hydrogen bond interactions may suggest possible reciprocal information transfer between oligopeptides [16] (S. Zhang, unpublished observations). Such oligopeptides could also be used as templates for the polymerization of nucleic acids. A proposed schematic diagram of such a hypothetical information transfer network is presented in Figure 3b and a similar proposal was presented by Crick [11]. Cairns-Smith had outlined the possible origin of prebiotic molecules and abiotic evolution in considerable detail [31–32]. It is plausible that once the primitive self-selecting and self-improvement information transfer systems were established, even if they had only a marginal efficiency, they could eventually evolve into a much more efficient and sophisticated system, such as the genetic coding system that ultimate-

ly took over. Metaphorically, our proposed simple and versatile coding system somewhat resembles the scaffolds that were once used for construction of ancient architectural structures, such as the Great Wall of China, the Partheon of Greece, the Colosseum of Rome, the Notre Dame de Paris and numerous medieval cathedrals. It was then necessary to first construct a simple scaffold before an elaborate structure could have been erected. Once the desired structures were completed, the scaffolds were dismantled and no trace was left of their existence. Such scaffolding in the origin of life has been postulated by Cairns-Smith in a clay-based prebiotic system [33]. Once the genetic coding system had evolved, it would eventually replace the clay-based information transfer scaffolding [33]. Likewise, the simple and versatile coding system proposed by our hypothesis and model is rudimentary and imperfect. Nevertheless, it is not unreasonable to speculate that such a coding system may have preceded the current much more sophisticated genetic coding system at an early stage during prebiotic molecular evolution in the origin of life.

Acknowledgment

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References

1 Watson, J. D. and Crick, F. H. C.: 1953, *Nature* **171**, 737–738.
2 Kornberg, A.: 1980, *DNA replication.* W. H. Freeman and Company, San Francisco.
3 Weissmann, C., Simon, L. and Ochoa, S.: 1963, *Proc. Natl. Acad. Sci. U.S.A.* **49**, 407–414.
4 Baltimore, D.: 1970, *Nature* **226**, 1209–1211.
5 Temin, H. M. and Mizutani, S.: 1970 *Nature* **266**, 1211–1213.
6 Brenner, S., Jacob, F., and Meselson, M.: 1961, *Nature* **190**, 576–581.
7 Gros, F., Hiattt, H., Gilbert, W., Kurland, C. G., Risebrough, R. W., and Watson, J. D.: 1961, *Nature* **190**, 581–585.
8 Heywood, S. M. and Rich, A.: 1968, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 590–597.
9 Nathans, D., Notani, G., Schwartz, J. H., and Zinder, N. D.: 1962, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1424–1431.
10 Crick, F. H. C.: 1958, in *The Biological Replication of Macromolecules. Symposium of the Society for Experimental Biology,* **Vol. XII**, pp. 138–163.
11 Crick, F. H. C.: 1970, *Nature* **227**, 561–563.
12 Zhang, S. and Egli, M.: 1994, submitted.
13 Barbier, B. and Brack, A.: 1992, *J. Amer. Chem. Soc.* **114**, 3511–3515.
14 Orgel, L. E.: 1972, *Isr. J. Chem.* **10**, 287–292.
15 Brack, A. and Spach, G.: 1981, *Origins of Life* **11**, 135–142.
16 Zhang, S., Holmes, T., Lockshin, C., and Rich, A.: 1993, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3334–3338.
17 Miller, S. L.: 1953, *Science* **117**, 528–529.
18 Miller, S. L. and Urey, H. C.: 1959, *Science,* **130**, 245–251.
19 Chothia, C.: 1973, *J. Mol. Biol.* **75**, 295–302.
20 Cohen, F. E.: 1993, *Science,* **260**, 1444–1445.
21 Walsh, C.: 1977, *Enzymatic Reaction Mechanism*, W. H. Freeman and Co., San Francisco, pp. 35–48.
22 Dyson, F.: 1985, *Infinite in All Directions*, Harper and Row, Publishers, New York, p. 92.
23 Offord, R. E.: 1989, *Semisynthetic Proteins*, John Wiley and Son, LTD., New York.

24 Corradin, G. and Harbury, H. A,: 1974, *Biochem. and Biophys. Res. Comm.* **61**, 1400–1406.
25 Offord, R. E.: 1987, *Protein Engineering.* **1**, 151–157.
26 Bartel, D. P. and Szostak, J. W.: 1993, *Science* **261**, 1411–1418.
27 Cech, T. R.: 1993, *Nature* **365**, 204–205.
28 Rich, A.: 1962, in *Horizons in Biochemistry*, M. Kasha and B. Pullman, (eds.), Academic Press, New York, pp. 103–126.
29 Kauffman, S. A.: 1993, *The Origins of Order*, Oxford University Press, NY p. 361.
30 Carter, C. W. and Krant, J.: 1974, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 283–287.
31 Cairns-Smith, A. G.: 1975, *Proc. Royal Society of London B*, **189**, 249–274.
32 Cairns-Smith, A. G. and Davis, C. J.: 1977, in *The Encyclopaedia of Ignorance*, Duncan, R. and Weston-Smith, (eds.), M. Wallaby, New York, pp. 391–404.
33 Cairns-Smith, A. G.: 1985, *Sci. Amer.* **252**, No.6, pp. 90–100.
34 Lancelot, G. and Hélène, C.: 1977, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4872–4875.
35 Lancelot, G., Mayer, R., and Hélène, C.: 1979, *J. Amer. Chem. Soc.* **101**, 1569–1576.
36 Rein, R., Garduno, R., Egan, J. T., Columbano, S., Coeckelenbergh, Y., and Macelory, R. D.: 1977, *Biosystems* **9**, 131–137.
37 Saenger, W.: 1984, *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, pp. 386–431.