EVOLUTION OF A REPLICATING PROTOCELL

ABSTRACT:
This model proposes a minimally constructed replicating protocell that exploits only a positive, a negative and a neutral amino acid to build membranes, genes and ion channels. This transition from chemical to biological evolution would result from a charged peptide that can function as a template to fuse peptide fragments, and act as a membrane gate.

The nucleic genetic code may have originated as a single base codon that recognized three types of amino acid residue. A two base codon with three base types could code for nine types of residue. An increase to four base types would produce 16 residue possibilities. The modern code now utilizes a three base codon and four base types to yield 20 types of amino acid. tRNA synthetases and the genetic code appear to be linked together by mutual evolution. The evolving transition to a nucleic code would support a greater variety of amino acids and proteins, and thus complete the creation of life.

Keywords: microsphere, ion channel, clay, matching salt bonds, synthetase, nucleic code, proteinoid

INTRODUCTION AND HYPOTHESIS

In the Beginning-the Peptide Code

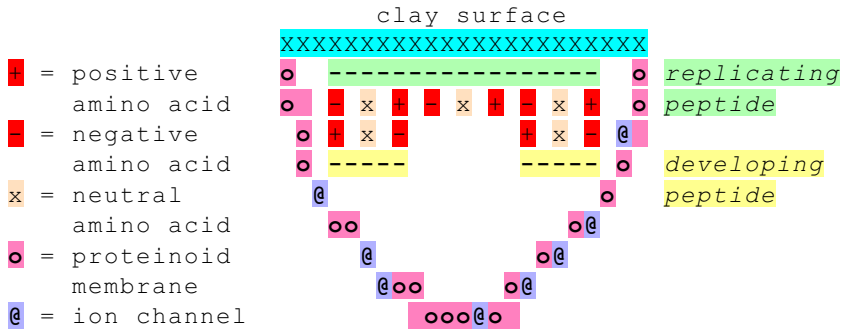
The primary elements of living cells are a reproductive system, a membrane to condense, eliminate and isolate cellular materials, and energy and molecular extraction. Proteins demonstrate enormous utility and flexibility by: 1- lengthening with amino-acid links; 2- forming numerous types of bonds-hydrogen, salt, covalent, hydrophobic; 3- enhancing many chemical reactions with these bonds and the shape of the protein. Protein involvement in cellular structures and enzymatic activities are well known. Simple amino acids can be heated to form membranes [1,4] and microspheres [1,4] with mild catalytic capacities [1,2,3,5]. These proteinoid microspheres can couple, fuse, bud, grow, form tubules, and transfer internal particles among this collective [4].

Thus the last required quality for a proteinoid protocell would be a protein based genetic code. Clay promotes peptide formation [6,7] up to 55 residues and 50 bases of RNA [8], and can also store and transfer energy [9]. Clay [10,11] and protein beta structures [12] induce amino acid and peptide enantiomers. Beta peptides constructed with alternating basic hydrophilic and hydrophobic residues can enhance homocirality as well as increase hydrolysis of RNA [13]. The decapeptide but not the hexapeptide exhibited these abilities [13]. A homochiral peptide replicator induces ligation upon similar enantiomeric peptide fragments [14]. Prions are infectious proteins that demonstrate proteic capacity for propagation [15].

Peptides can replicate by utilizing an anti-parallel stance [16,17] which also can form transmembrane ion channels [18,19]. These examples can be useful to envision positive (such as lysine or ornithine), negative (such as aspartic

acid), and neutral (such as glycine or alanine or valine) amino acids to fashion a hypothetical peptide (fig. 2). Microspherical membranes are formed with a simple heated mixture of glycine, valine, alanine, aspartic acid, and salts [1] which are abundantly generated in abiotic synthesis [20 p.88-95] as well as lysine and ornithine [34 p.137-62]. Lysine can be substituted for aspartic acid in membrane formation [1]. Silicate (the main component of clay) binds microspherical membranes with proteinoids that contain about 10 to 20 residues [21]. A 16 residue peptide consisting only of alternating positive, negative and neutral amino acids constructs membranes by matching salt bonds [22]. Peptide replication utilizing positive-negative pairings and hydrophobic matches has been suggested [20]. Tri-peptides are created in modest quantities with abiotic conditions [21A].

Figure 1 **Proteinoid Protocell**



This protocell could contain by chance from microsphere creation tri-peptides and a nine residue peptide (fig. 2) to spark life that is similar to charged tri-peptides forming nine residue peptides upon a mineral surface [22A]. Charged peptide fragments and clay create 45 residue peptides [23] as well as charged peptide coils function as a template to align peptide fragments with salt bonds that assist in peptide fusion [24]. Darwinian survival should be enhanced in protocells with similar peptide repliants (fig. 1).

Only a single charged tri-peptide would properly align on the progenitor peptide in three binding regions (fig. 3A). Tri-peptides without this correct sequence could not bind on the template. Normal twisting around the alpha carbon bonds of the replicating peptide backbone would allow the two ends of the tri-peptide pair to align adjacent (fig. 3B). Positive and negative charges on the clay surface [25] would temporarily stabilize the 180 degree rotation of the replicating peptide at the N and C terminals, and enhance fusion of the tri-peptide pair (fig. 3C).

Figure 2

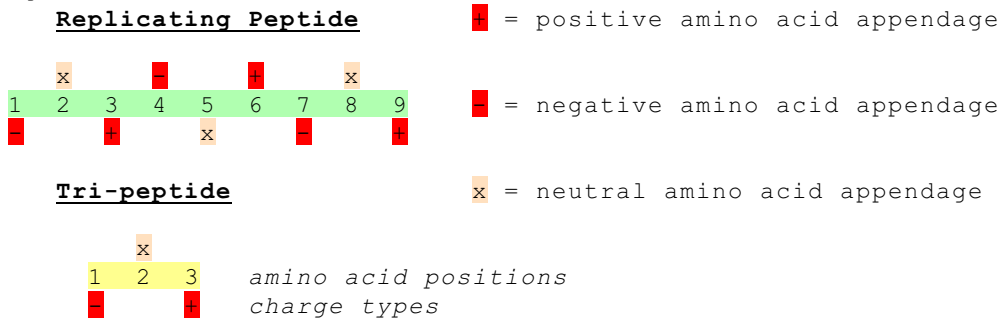


Figure 3A **Peptide Reproduction**

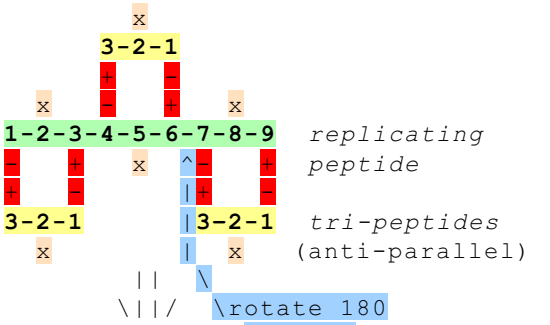


Figure 3B

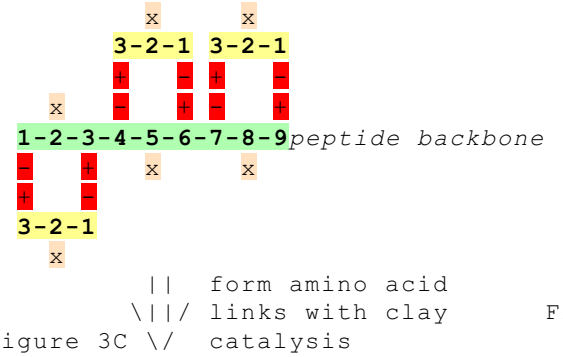


Figure 3C form amino acid links with clay catalysis

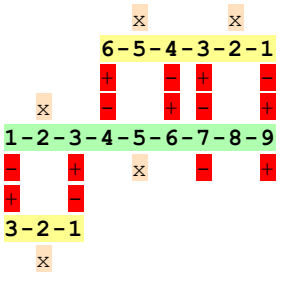


Figure 3D repeat tri-peptide fusion

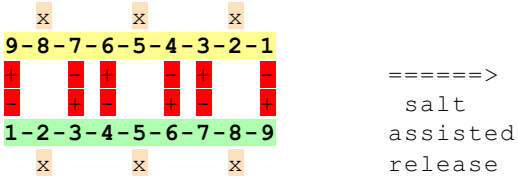


Figure 4A **Peptide Coil**

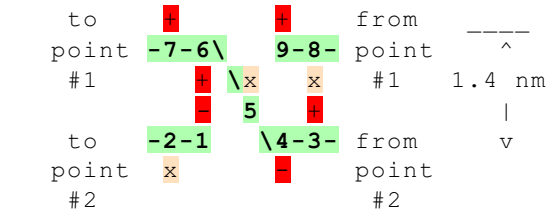


Figure 4B

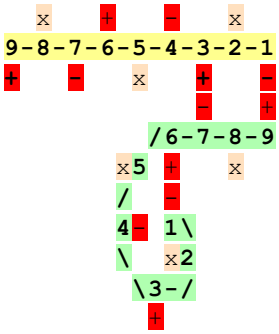
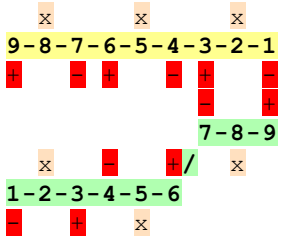


Figure 4C coiling



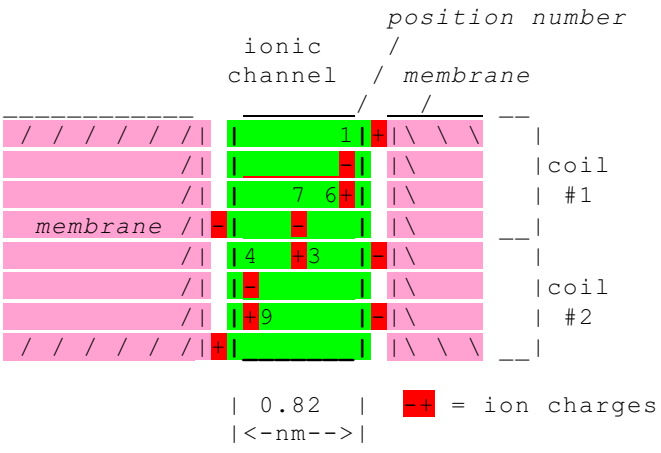
Cellular salinity could assist in the release of the newly minted peptide (fig. 4C) and inhibit ligation [26]. Two fates are possible for this peptide depending upon salt levels that influence the configuration assumed. If the peptide maintained an alpha structure without salt bonds then another peptide gene would be created. In the event of diminished salinity that fosters beta peptides to coil into channels [27], a salt bridge might be establish between positions 1 and 6 or 4 and 9 (fig. 4A). Many peptides with beta structures develop into membrane channels [28].

The interior and exterior of this coil would display polarized oxygen and hydrogen appendages. The coil with an outer diameter of about 0.82 nm and an inner diameter of about 0.32 nm might be inserted into a polarized region of the membrane as the protocell ages and extrudes the interior contents [4]. Two or more coils may fuse with salt bridges at positions 3 and 7 and extend the length of this tubular structure (fig. 5). This transmembrane ionic channel could exclude non-polar peptides and allow passage of charged forms. Self-assembling transmembrane peptide channels can transport charged amino acids, and display electrical oscillations similar to neural ionic membrane channels [29].

Microspheres created with arginine, aspartic and glutamic acids can rhythmically depolarize and repolarize membrane charges that is extremely similar to neural ionic membrane channels when conducting action potentials [30]. This ionic fluctuation may alternate peptide dimer separation and formation (fig.3), and effect coiling (fig. 4).

Compartmentalized microspheres arise with the addition of lysine rich proteinoid in salt solution into a broth containing aspartic rich microspheres, aspartic-glutamic rich proteinoid and salt [30A]. Increased compartmentalized microspheres also result from rising concentrations of salt in a mixture of aspartic-glutamic rich proteinoid and lysine rich proteinoid solutions [30A]. These observations may indicate that as a protocell incorporates more ion channels into the membrane, the influx of salt and ionic peptides induce cell division. The following growth of the progeny and infusion of new proteinoid [4] should lower cell salinity until growth naturally ceases [4] and more ion channels are created.

Figure 5 **Transmembrane Channel**



An alternative scheme to produce a wider coil with an inner diameter of about 0.9 nm would utilize an anti-parallel dimer. This dimer would be fused with four salt bridges with the interface at two dual positions of 7 to 9 and 1 to 3 (fig. 6A). Multiple coils could ionically mesh at the dual positions 4 to 6 (fig. 6B) and produce a transmembrane tubular ionic channel.

Figure 6A **Anti-parallel Dimer**

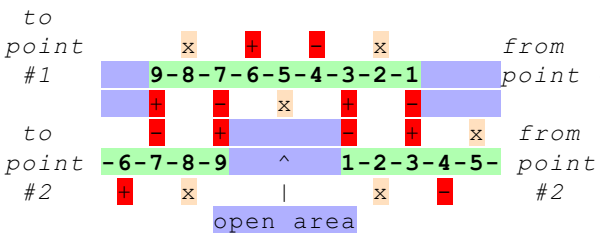
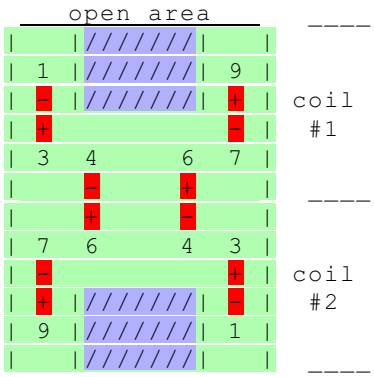


Figure 6B **Dimer Coil Tube**



Point mutations at neutral sites on the peptide gene may be important. The fundamental utility as a duplicator and channel should continue while favorable enzymatic enhancements might be possible. Peptides formed under prebiotic conditions that are rich in lysine demonstrate modest aminotransferase activity [2]. Biology might now begin.

This concept of peptide replication can be evaluated by mixing salts, clays, and nine reside polar peptides with labeled polar tri-peptides. Gel electrophoresis can then be utilized to search for labeled nine residue peptide repliants. Further examination with this method and proteinoid microspheres may thus yield an extremely primitive replicating protocell.

Darwinian Transition to a Nucleic Code

The phosphoribose backbone may have evolved before the nucleosides by developing the utility of energy storage and transfer when cleaving a ribose phosphate. Evolutionary advances in peptide synthesis might use primitive enzymes to attach ribose phosphates to amino acids that is similar to modern synthetases [31,32]. These activated amino acids would selectively adhere to specific residues of the proto-synthetase by utilizing hydrophobic, salt, hydrogen bonds, and the shape of the synthetase. These amino acids could be oriented upon the synthetase template with hydrogen bonds, and stabilized with hydrogen bonds between themselves. The phosphate group might transfer the activated amino acids (R1, R2) to a thioester of a cysteine like residue on the synthetase template (fig. 7A). The thioester linked amino acids (R3, R4) might act as a zipper to exchange the sulfur for a peptide bond to create a new peptide (fig.7B). This model is based upon non-ribosomal peptide synthetases that bind aminoacyl adenate and weld them into peptides via thioesters without nucleic acids [32]. Common minerals can condense phosphate into polymers via thioesters [33] which can be used to activate amino acids and produce peptide formation [34].

Figure 7A **PROTO-SYNTHETASE**

aminoacyl ribose phosphate exchange with thioester

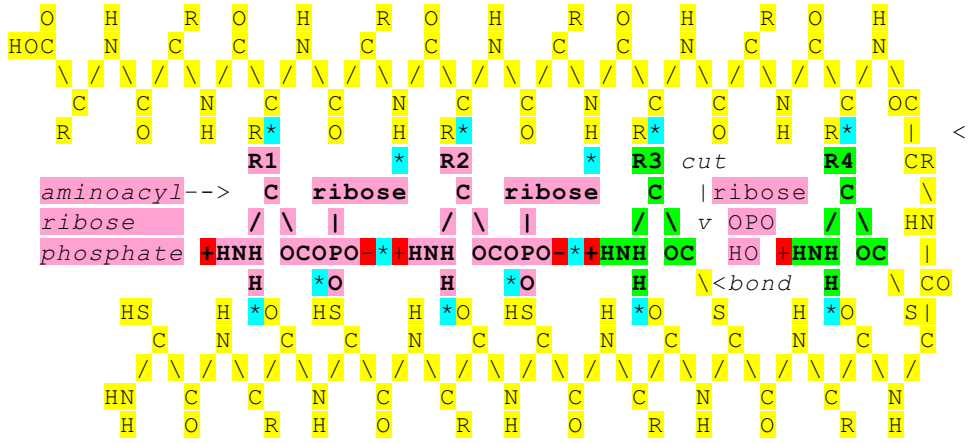
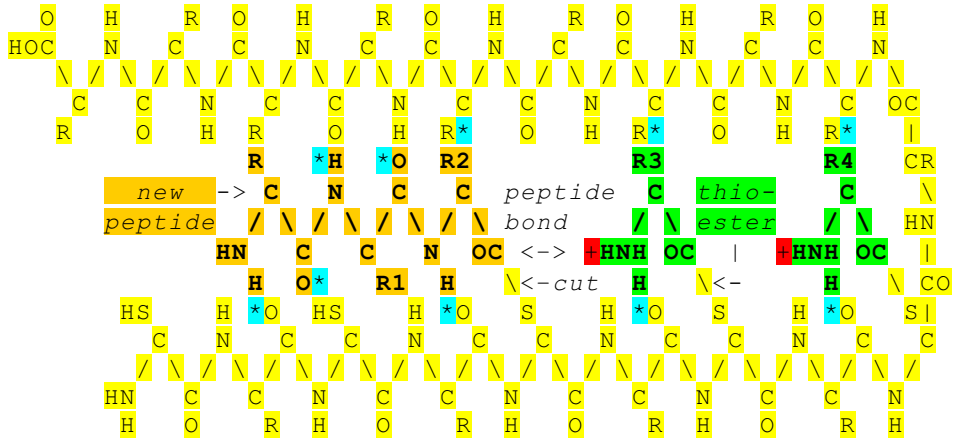


Figure 7B

|< 0.67 nm >|

|| peptide formation
\||/ from thioester zipper
\/

S = sulfur
P = phosphorus
C = carbon
H = hydrogen
O = oxygen
N = nitrogen
R = random appendage



Nucleic acids are rather difficult to synthesize abiotically [20] when compared to peptides which indicates that biological creation began with peptides. Nucleic origins might have started with phosphoribose strands that incorporated various nucleosides to help regulate proteinoid membrane functions. Dinucleotides selectively associate with amino acids (and perhaps proteinoids) that have similar hydrophilic nature [50] which might modulate the hydrophobic spectrum of various membrane regions. The fluidity of the membrane could be influenced by polymerization of nucleic or peptide strands. Alpha helices and beta sheets utilizing hydrogen and salt bonds among peptide and nucleic appendages could contribute to viscosity properties. Short RNA stands can associate with the membrane to enhance passage of charged ions [35]. Phospholipids could then be utilized to seal hazardous leakage from the proteinoid membrane structure [36].

Nucleotides might then specialize in mutual genetic recognition (***C-G***,***A-T***, ***A-U***) that would replace the inferior salt bond discrimination of peptide genes. Zhang and Egli [37] proposed that peptides and RNA could reciprocally encode for each other by hydrogen bonding. Two hydrogen bonds would link guanine (***G***) with negative charges on aspartic (asp) or glutamic (glu) acids, cytosine (***C***) with positive charges on lysine (lys) or arginine (arg), and adenine (***A***) or uracil (***U***) or ***C*** or ***G*** with bipolar charges on asparagine (asn) or glutamine (gln) (fig. 9A). The ***A*** or ***U*** would be superior to ***G*** or ***C*** when binding to asn or gln because the base and residue charges are balanced the same. This proposition can be simplified by ***G*** with asp, ***C*** with lys, and ***A*** with asn (fig. 9B). Lysine-rich proteinoid binds with polycytosine while arginine proteinoid does not [38].

The peptide would serve as a template to create the RNA molecule. This molecule might be utilized to synthesize copies of the structural form of the peptide while the original peptide gene can be reserved for the manufacturing of more peptide genes and RNA. Microspheres created with positive and negative amino acids, and ATP form polynucleotides [39] as well as polypeptides [40]. Different homopolynucleotides in nucleoproteinoid particles will foster different peptides [40]. Amino acid enantiomers selectively bind with nucleic enantiomers [41] which indicate that D type RNA may have been favored from interacting with L type protein.

The primary tools in modern protein synthesis are mRNA codons which transfers the code from DNA, tRNAs which matches its anticodons with mRNA codons and fastens to amino acid residues, and tRNA synthetases which recognize individual tRNAs and their amino acids to unite them. The first base of the codon may represent the original three residues. The codon's second base clumps together corresponding amino acids with similar chemical properties as would be expected if a major expansion of the code occurred with the inclusion of the second base. The ***U*** associates with large hydrophobic residues. The ***A*** associates with hydrophilic charged residues.

The tRNA types do not appear to relate to this peculiar codon clumping [42] where as synthetases demonstrate similar groupings. Synthetases are classified into types I and II [43,44]. Type I is subdivided into classes with hydrophobic residues in IA and aromatic residues in IB that are coded with ***U*** as the first or second codon base, and the larger charged residues in IC. Type II is subdivided with hydroxyl and penta-ringed residues in IIA, smaller charged residues in IIB (the original residues in fig. 9B), and the smallest residues in IIC. Phenylalanine synthetase has characteristics of both type I and II.

The amino acids in synthetase classes IIA and IIC share the same first base of the codon when they are associated together by chemical structures (fig.9C). The residues in synthetase classes IA and IC primarily utilize the second base of the codon when their chemical properties are matched together. Class IB synthetases appear to have evolved last [45]. These observations lend credence to the notion that type I synthetases appeared after type II [46,47]. The type II synthetases appear to be related to non-ribosomal peptide synthetases that do not utilize nucleic acids [48].

If the codon is simplified to two bases with four base types, and the synthetase divisions are factored in (removing class IB and IC residues that overlap classes IA and II) then the genetic code can be depicted as in figure 11. When uracil and class IA synthetases are eliminated, the genetic code is reduced to allow the residues in figures 9B and 9C to combine in figure 10. Several sequential conclusions can be deduced from this data (table 1).

Table 1 **Genetic Code Progression**

step A-one base per residue- first RNA
three base types (***A,G,C***)
three residue types(asp, asn, lys)
fig. 9B

step B-two base anticodon
three base types (***A,G,C***)
nine small and hydrophilic
residue types fig. 10

step C-type II synthetases

step D-DNA three base types (***C,G,T***)
tRNA three base types(***C,G,U***) fig. 12
mRNA three base types(***C,G,A***) fig. 12

step E-two base codon
four base types
mRNA and tRNA (***C,G,U,A***)
four base types DNA(***C,G,A,T***)
15 residue types and end
fig. 11

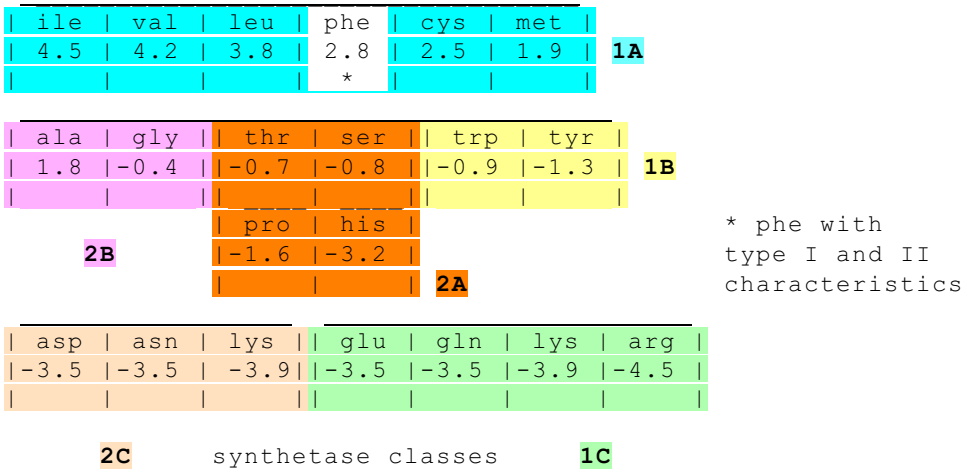
step F-class 1A synthetases

step G-three base codon
four base types
mRNA and tRNA (***C,G,U,A***)
20 residue types
class IB and IC synthetases

The original peptide-nucleic code contained only three charged residues and three base types {step A}. The code could then be expanded to two bases that selectively associate with nine small and polarized amino acids {step B}. The wobble factor on site 34 of the tRNA argues for an earlier anticodon that utilized only two bases [49]. Hydrophilic values of the amino acids in figure 10 linearly correlate with hydrophilic levels of the corresponding dinucleotide anticodons [50]. This correlation is more pronounced for the second base which would be expected if the genetic code progressed to a two base anticodon. The first RNA might have used anticodons which may have been inverted to mRNA codons when tRNA assumed the anticodon-synthetase interface {step D}. Each amino acid would have some special affinity for its individual dinucleotide. This system while producing advanced proteins would certainly have many translation errors.

The hydrophobic values of the 20 modern amino acids seem to clump together in a very similar manner to synthetase classes (fig. 8) [51] which supports the idea that physical properties of amino acids early in the evolution of life molded various synthetase divisions.

Figure 8 **Hydrophobic Values of Amino Acid Correlate with Synthetases**



Primitive type II synthetases would be advantageous to minimize binding defects and enhance specific anticodon-residue alignment {step C}. Proteins would cease any coding function. To improve upon nucleic code translation, DNA with thymine (***T***) would specialize as the master replicator and space efficient information storage while tRNA with uracil would interface between the RNA messages and residue recognition of synthetases {step D}. Small RNA can foster peptide bonds from aminoacyl adenate [52] that maybe a pathway toward proto-ribosomes and proto-tRNA.

The two base codon could then incorporate uracil to expand to four base types and 15 residue types (fig. 11){step E}. The added amino acids would be primarily hydrophobic (phe, cys, leu, ile, val) and a special signal to terminate the protein (end). This expansion would necessitate the inclusion of type I synthetases {step F} which some have been reported to have appeared after tRNA [53]. Interestingly modern exceptions to the genetic code involve codons with ***U*** as the first or second base, and the arginine-lysine codes [54]. Lastly the code can be enhanced to 20 types of amino acid by enlarging the codon to three bases per residue {step G}, and arginine usurping lysine and some serine codons [55,56,57]. Ornithine has been suggested as the usurped residue [57] which would also indicate that lysine must have assume the ornithine synthetase as well as some asparagine codons. This may explain why lysine has two types of synthetases [53].

Figure 9A

Selective Nucleotide-Amino Acid Binding

nucleotide *amino acid*

[+-+] ***G*** <--> [-] asp glu

[-+-] ***C*** <--> [+] lys arg

[+-] ***A U*** <-> [-+] asn gln

charge

Figure 9B

(simplified)

[+] ***G*** <--> [-] asp

[-] ***C*** <--> [+] lys

[+-]***A*** <--> [-+]asn

1 base per residue

Figure 9C

Chemical Properties of Amino Acids Correlate with Codon

residue	codon	properties
gly	***GG***	
ala	***GC***	smallest
pro	***CC***	nitrogen
his	***CA***	penta-ring
ser	***AG***	hydroxyl
thr	***AC***	group

Primitive Genetic Code

Figure 10
2 bases per residue codon

	C	***A***	***G***
C	pro	his	lys
A	thr	asn	ser
G	ala	asp	gly

2nd base

======> 1st base

modern genetic code <======
for 20 amino acids,
3 bases per residue codon

Figure 11
2 bases per residue codon

	U	***C***	***A***	***G***
U	phe	ser	end	cys
C	leu	pro	his	lys
A	ile	thr	asn	ser
G	val	ala	asp	gly

E. Coli synthetase classes differ by their capacity to recognize specific points on individual tRNAs (table 2) [60,61]. These points are predominately located at the 1st and 2nd base (sites 35, 36 and some 34 of the 3rd base) of the anticodon and the terminal stem. Each synthetase may also utilize other tRNA regions that are not shared by the majority.

Synthetase classes 2C, 1A, 1B and phenalanine primarily recognize the anticodon and terminal site 73. Classes 2A and 2B mostly select the terminal sites 73, 1-72 and 2-71. Class 1C mainly utilizes the anticodon in conjunction with sites 73 and 1-72.

Within each synthetase class, only a portion of the recognition sites appear to discriminate among individual members (note bold and underlined nucleosides in table 2). Classes 1A, 2C, and phenalanine rely on the anticodon (met and ile also use site 34). Classes 1C and 1B utilize the anticodon along with sites 73 and 2-71 respectively. Classes 2A and 2B use different terminal sites for discrimination (note similarities with Fig. 9C).

Type 1 synthetases thus differ from type 2 (except 2C) by enhanced reliance upon the anticodon while the later relies upon the terminal stem. Each synthetase class seems to have developed special patterns that created a secondary code to recognize specific tRNA which may reflect upon the origin of tRNA and the genetic code.

The tRNA partners of 1B and 1C synthetases have very few tRNA sites that are universally shared among all branches of life (eucarya, archaea, bacteria) [42] which may indicate the late arrival of these classes. Universally conserved spots are maximized at the terminal site 73 (55% of tRNAs) and progressively diminishes further down the terminal stem (1-72=50%, 2-71=30%, 3-70=15%) [42].

Table 2 **E.Coli Synthetase-tRNA Recognition Sites** [60,61]

synthetase class	amino acid	tRNA Terminal Stem: 73	1-72	2-71	Anti-codon 35-36	3rd base 34
	his	***C***	***G-C***		U-G w	w
	pro	***A***	***C-G***		G-G	N
2A						
	ser		G-C	***G-C***		N
	thr		G-C	***C-G***	G-U	N
	gly	***U***	G-C	***C-G***	C-C	N
2B	ala	***A***	G-C	***G-C***		N
	asp	G	G-C w	G-C w	***U-C***	Y
2C	asn	G			***U-U***	***Y***
	lys	A			***U-U***	***Y***
	phe	A w			***A-A***	Y
	cys	U		G-C	***C-A***	Y
	met	A		G-C	***A-U***	***Y***
1A	ile	A			***A-U***	***Y***
	val	A			***A-C***	N
	leu	A				N
	arg	AG w			***C-G***	N
	gln	G	U-A	***G-C***	***U-G***	Y
1C	glu	G	G-C	***U-A***	***U-C***	Y
	lys*	A		***C-U***	***U-U***	Y
	tyr	***A***	G-C		***U-A***	N
1B	trp	***G***			***C-A***	Y

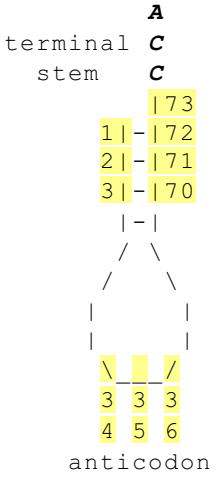
w = weak

Y = yes

N = no

* = [65]

Simplified tRNA



Adenine is always the final base on the tRNA terminal stem that directly attaches to an amino acid and to the ribosome [62]. A glutamic acid rich ribosomal protein appears to rotate to advance tRNA and has electrostatic repulsion between the protein and tRNA [63]. A proto-ribosome can be imagined by aligning a proto-tRNA upon a mRNA template (fig. 12). Two bases at the nadar of a hairpin loop of the tRNA would bind as the anticodon, and the adenine terminal would grasp the amino acid with the assistance of a synthetase. The adenine base displays a positive and a negative site for hydrogen bonding to asn [37]. Two tRNAs could be aligned adjacent to each other at the anticodon by the mRNA and at the terminal stem by a penta-peptide composed of asn and glu. The two asn would form two hydrogen bonds with each adenine while glu could enzymatically foster the peptide link [64] (fig. 13A). The glutamic #3 carboxyl group of the penta-peptide strips an electron from the amino terminal of the aminoacyl-tRNA which with glu#2 severs the peptide link at the ester junction of the peptidyl tRNA and fosters the elongated peptide from the former aminoacyl tRNA. When the peptide bond is formed a hydroxyl negative charge is left on the empty tRNA terminal which could repel the negatively charged glu#2, and cause asn#1 to swing into a new position with asn#2 at the center of the pivot. Asn#1 could assist in aligning a new tRNA (fig. 13B). The empty tRNA would no longer have a terminal support, and glu#3 would swing around to expel the empty tRNA from the mRNA site.

Figure 12 **PROTO-RIBOSOME**

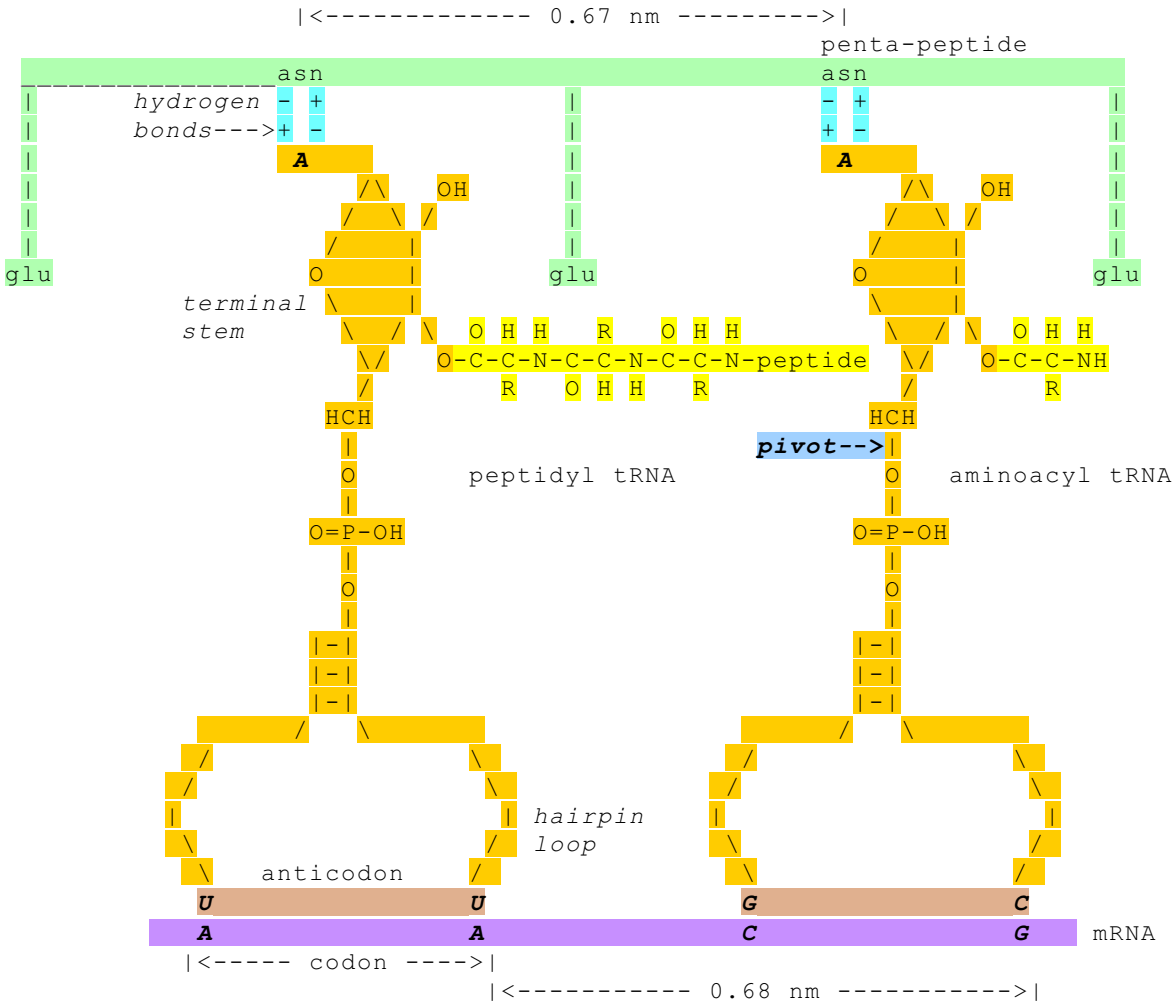


Figure 13A **Peptidyl Transferase Reaction**

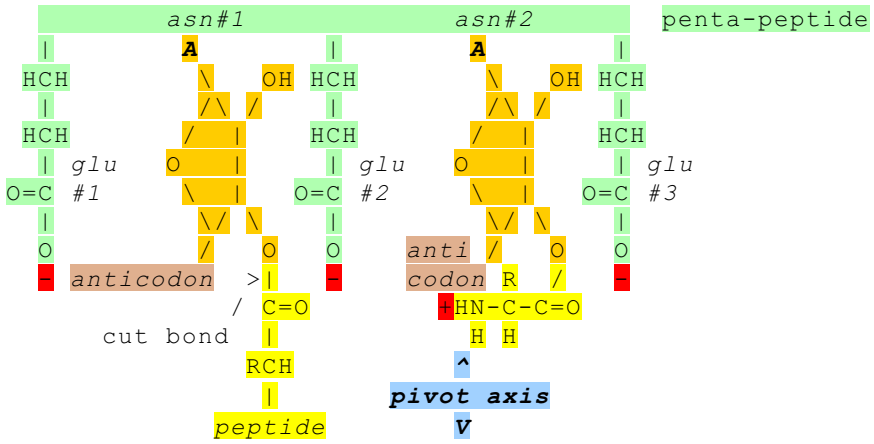
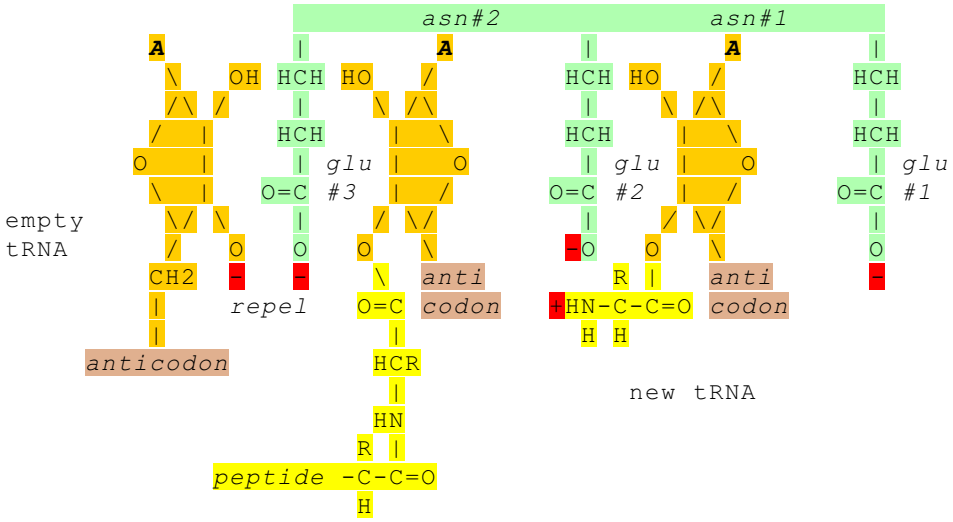


Figure 13B



DISCUSSION:

The general strategy of this article is to reverse engineer modern genetic systems, and simplify models to describe plausible primitive mechanisms. The key to theorizing about the distant past of life is to understand and correlate the essential functions of cellular reproduction that has continued through billions of generations. The principle of this essay is not to proclaim the pristine pathway to the genesis of life but to demonstrate that a few amino acids could progress through discrete and observable steps toward a simplistic reproducing cell. A similar process might also utilize RNA or other polymer templates. This proteinoid protocell need not be efficient but just stable enough to generate more offspring than molecular decay can eliminate. In a static environment such as deep sea vents [1], these primitive cellular structures could enjoy a reasonably long existence lasting years [4 p.41]. No biological competition existed.

The mutual evolutionary influences between mRNAs and synthetases may have provided an exponential yield of amino acid specialization as well as genetic code improvements with DNA and tRNA enhancements. The development of a peptide-RNA interface would permit the utilization of many more types of amino acid which would allow for more sophisticated proteins. These proteins would provide the cellular mechanisms for advances in replicating efficiencies, membrane structures, and metabolic capacities.

Life probably began after 3.9 billion years ago when bombardment of the earth ceased [66]. Firm evidence of life dates from 3.46 billion years ago [67,68,69,70,71], and microfossils and biochemical traces that are 3.8 billion years old have credibility [72,73,74] where as 3.85 billion year old samples are probably abiotic [75]. Molecular studies [76] indicate that archeae and bacteria diverged about 3.125 billion years ago. This indicates that most genetic mechanisms had evolved by this point except for refinements such as type I lysine synthetase [53]. The peptide genetic code may have arisen within several million years and the nucleic transition might have taken hundreds of millions of years.

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This natural history is dedicated to the historian Cameron Stewart.