A THEORY OF THE ORIGIN OF LIFE

CHARLES B. OLSON**

Dept. of Biological Sciences, Stanford University, Stanford CA, U.S.A.

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Abstract. Life on Earth is essentially nucleic acids (NAs) influencing peptide synthesis such that NA replication is favored. It is proposed that the ability to synthesize polypeptides evolved gradually – one peptide bond at a time. The proposed evolution of the peptide synthesis apparatus begins with a 'transfer NA' (tNA) which catalyzes the transfer of activated amino acids to accessible amino groups in its environment. The resulting 'capped molecules' (with single amino acid 'caps') in turn favor NA replication. The proposed evolution of the peptide synthesis apparatus from the tNA onward is characterized by a progressive increase in the number of amino acids per cap: two tNAs jointly produce a 'dipeptide cap', three tNAs jointly produce a 'tripeptide cap', etc. Messenger NAs evolve because they can specify the composition and sequence order of the peptide caps. Lastly, ribosomal NAs evolve. The origin, expansion, and standardization of the genetic code are discussed. It is proposed that the present triplet code evolved by a process of codon length refinement, and that originally codons of varying lengths were allowable, as were unassigned bases between codons. An environmental supply of activated compounds for early evolving entities is proposed. An environmental NA replication process via single template-directed bond formation events is proposed. An 'environmental retention and redistribution process' is proposed to have acted as a functional substitute for the cell wall and cell division of early evolving entities.

1. Introduction

The origin of life is the moment when evolution began. Evolution is inextricably linked to selection, and selection is inextricably linked to both the ability to replicate and the ability to mutate (the ability to undergo a heritable alteration); the ability to evolve entails both of these abilities. It is assumed that from the earliest evolution onward, the chemical basis of heredity was the replication of nucleic acids* (NAs) made possible by their unique ability to hybridize via complementary base pairing (Orgel, 1968). The main justification for this assumption is continuity: 'the replication of nucleic acids is the central reaction responsible for the transmission of hereditary information in all contemporary organisms' (Orgel and Lohrmann, 1974).

Much of this paper can be included in two large subdivisions which perform two major tasks. The first task is to propose how the primordial environment could perform the processes essential to life (energy supply, NA replication, retention (cell wall), and redistribution (cell division)); this is done in the sections entitled 'Environmental energy supply', 'Environmental nucleic acid replication', and 'The environmental analogues of the cell wall and cell division'. The second task is to propose an

* Nucleic acids (NAs) in the primordial soup are not specified as RNA or DNA since NAs in the primordial soup presumably contained a variety of sugars in their backbones; indeed any molecule which would hold the bases together and allow NA hybridization would be functional (Orgel, 1968). The primordial soup is assumed to contain, among many other things, a great variety of peptides and NAs (Oparin, 1964; Orgel and Lohrmann, 1974).

** Present address: 416 Leland Avenue, Palo Alto, CA, 94306, U.S.A. This paper is dedicated to truth, beauty, and love.

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evolution of the translation apparatus; this is done in the sections entitled 'Mainstream evolution I: the tNA', 'Mainstream evolution II: tNA-mNA', and 'Mainstream evolution III: tNA-mNA-rNA'.

2. Environmental Energy Supply

The most fundamental biological reactions (NA replication, peptide bond formation, and the activation of tRNAs with aminoacyl adenylates) are transfer reactions, whereas dehydration reactions are rare in biological systems simply because in an aqueous environment they are thermodynamically unfavorable** (in contrast, see Woese, 1979). It is proposed that throughout the earliest stages of evolution, biological reactions (those reactions in which evolving entities participate, such as NA replication or NA-mediated peptide bond formation) were predominantly or exclusively transfer reactions; and therefore the early evolving entities were heterotrophs (Horowitz, 1945). These heterotrophic entities required a continuous source of 'energy' – of dehydrated ('activated') compounds – analogous to an ATP source for modern cells. This 'environmental energy supply' could result from the particular features of the local landscape. As shown in Figure 1, evolving entities which are

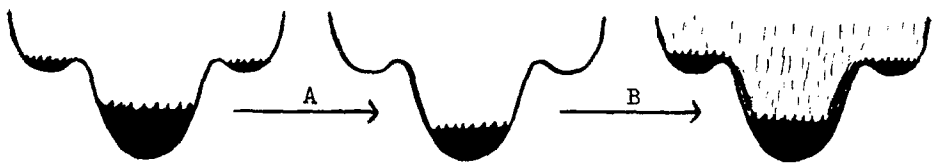

Fig. 1. The environment provides a supply of activated compounds to evolving entities in an aqueous environment (the central pool). A. Peripheral pools evaporate completely, and dehydration reactions occur; B Rain dissolves the dehydration reaction products and carries them into the central pool. Note that the central pool does not dry up or overflow at any point in this process.

permanently located in a relatively deep 'central pool' (which neither dries out nor overflows) could receive activated compounds from nearby 'peripheral pools' which (1) are shallow relative to the central pool and dry out at least occasionally; and (2) are at a higher altitude than the central pool such that when they fill with rain, the soup (with dissolved dehydration reaction products) overflows and runs downward into the central pool. Similarly, Gibbs *et al.* (1980) have proposed that long oligonucleotides could have been protected from environmental cycles of wetting and drying by selective adsorption on permanently submerged mineral surfaces such as hydroxyapatite. An important advantage of their proposal relative to this one results from the ability of the pool with the submerged mineral surfaces to overflow without losing its adsorbed long oligonucleotides. Potential biological substrates such as deactivated mononucleotides, short oligonucleotides, and amino acids may thus leave the pool, be reactivated, and subsequently return to the pool.

** The major exception is the phosphorylation of ADP to ATP in mitochondria and chloroplasts (a dehydration reaction).

3. Environmental Nucleic Acid Replication

The proposal that during the earliest evolution, NA replication was strictly environmental (i.e. without the aid of biologically-produced catalysts) is not new: a great deal of experimental work and discussion has taken place (Orgel, 1968; Naylor and Gilham, 1966; Lohrmann and Orgel, 1979; Ninio and Orgel, 1978; Orgel and Lohrmann, 1974; Sulston *et al.*, 1968; Woese, 1972). The proposed evolution of the translation apparatus which is described later in this paper does not depend upon the specifics of the environmental NA replication process. However, I would like to briefly sketch my own views on this important area.

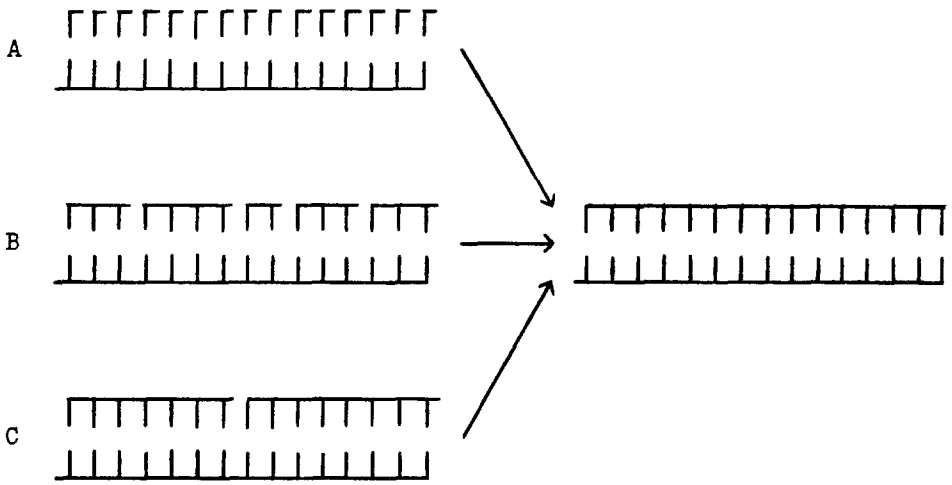

Fig. 2. Replication of a NA of length 15 by means of three different environmental NA replication processes. A. 15 mononucleotides are joined by 14 template-directed bond formation events; B. 5 short oligonucleotides are joined by 4 template-directed bond formation events; C. Two longer oligonucleotides are joined by a single template-directed bond formation event.

Previous proposals for environmental NA replication call for multiple template-directed bond formation events between many mononucleotides or short oligonucleotides (see above references) as shown in Figure 2A, B. An implicit assumption of these proposals is that the environmental catalysis of template-directed bond formation was efficient enough to form several bonds within the lifetime of a hybrid. There is a simple alternative which makes this assumption unnecessary: an environmental NA replication process based on single template-directed bond formation events (see Figure 2C). This process has the relative advantage that the longer hybrids are more stable (with longer lifetimes) and would exist under a greater range of environmental conditions; and it has the relative disadvantage that the longer oligonucleotides it uses as substrates would be present in much lower concentrations than shorter oligonucleotides or mononucleotides. A crucial advantage of the process of Figure 2C is that only a *single* template-directed bond formation event must occur during the

lifetime of the hybrid. The probability of multiple bond formation events occurring within the lifetime of a hybrid decreases exponentially as their number increases. Certainly the rate of template-directed bond formation reaction when catalyzed only by the environment (without even a primitive biological catalyst) would be expected to be quite low. This low rate would be made even lower by the presumably relatively high concentration of deactivated substrates and other substances which could block the reaction. These considerations favor single bond formation events relative to multiple events.

Another important consideration is how changes in environmental conditions would be expected to affect the frequency with which the different types of hybrids in Figure 2 tended to form. Temperature changes were presumably the most important. Because early evolving life could be expected to evolve in pools of a size large enough to rarely or never dry up, and because water cools slowly due to its large heat capacity, it is proposed that gradual decreases in temperature tended to favor formation of hybrids of the type in Figure 2C relative to those in Figure 2A, B.

4. The Environmental Analogues of the Cell Wall and Cell Division

A cell wall performs an essential function for NA(s) which alter molecules in the environment such that NA replication is favored: it prevents the favorably altered molecules of the environment and the NA(s) from diffusing away from each other. This is referred to as 'retention', and there are two fundamentally different ways to 'retain' the NA(s) and the altered molecules of the enivironment:'direct retention' and 'indirect retention'. Direct retention is when the NA(s) and the altered molecules are directly or indirectly linked together (by covalent and/or noncovalent bonds). Indirect retention is when the NA(s) and the altered molecules are free to diffuse about but are inside a (relatively small) 'isolation chamber' which physically blocks their exit from the chamber. The isolation does not have to be 'complete' (with impermeable barriers) – it may be 'partial' (with selective permeability for some classes of molecules, e.g. those below a certain size) as long as the NA(s) and the altered molecules are retained within the chamber. The 'retention set' of a NA refers to the set of molecules which collide with the NA at a relatively high frequency due to physical links (direct retention) and/or physical barriers (indirect retention).

Early in evolution, it is proposed that retention of evolving NAs was predominantly or exclusively environmental (i.e. not biological: not influenced by a heritable trait of an evolving entity). It is proposed that the early stages of evolution took place in an environmentally-formed thick loosely-packed layer of tangled organic polymers (e.g. polypeptides, NAs, polysaccharides, and heteropolymers) which was located on the bottom of a pool which was situated such that it would receive and accumulate activated compounds from nearby pools at higher altitudes (see the central pool in Figure 1). This organic layer would presumably be a random labyrinth with chambers and passageways of a great range of sizes and shapes distributed through-

out, thus randomly providing various NAs (which are attached or unattached to the matrix) with a variety of retention sets.

The division of a cell into two cells performs the essential function of giving NAs with a common retention set two separate retention sets such that the two newly independent groups of NAs can each favor NA replication according to their own respective abilities. Evolution cannot proceed without this essential 'redistribution' process which gives the 'progeny NAs' of a successfully replicated 'parent NA' their own retention sets such that selection can operate among them.

Early in evolution, it is proposed that redistribution was predominantly or exclusively environmental. For NAs retained in the proposed tangled organic layer, environmental redistribution might occur occasionally on a small (molecular) scale via diffusion, fortuitous hydrolyses or bond formations, chance events of molecular tangling or untangling, etc. In addition, it is proposed that environmental redistribution was greatly aided by periodic large-scale environmental disruptions of previous retention sets (e.g. by rain or currents) such that NAs with overlapping or identical retention sets would subsequently have separate retention sets. The periodicity of these large-scale environmentally-caused redistributions might be anywhere from very frequent (e.g. daily) to very rare (yearly or less frequent), depending on such factors as the frequency and seasonal nature of rainfall and fluctuations in the depth of the pool.

The total process, encompassing the essential functions of both the cell wall (retention) and cell division (redistribution) shall be called the 'environmental retention and redistribution process'. Because this process is environmental, it allows entities to evolve without having to possess their own functional equivalent of a cell wall and cell division process from the outset.

5. Mainstream Evolution I: The tNA

The goal of the preceding sections was to outline how NAs could have been replicating and mutating – evolving – in the primordial environment. It is proposed that the first major step in evolution was the formation of a 'transfer NA' (tNA) which favored its own replication by altering molecules in its retention set: it possessed the ability to (slightly but significantly) catalyze the transfer of activated amino acids (activated at the carboxyl group) to accessible amino groups (e.g. on the chamber wall) by means of a covalent intermediate involving the carboxyl group of the amino acid and a hydroxyl group of the tNA as shown in Figure 3. Through this *single* bond formation, the proposed tNA favored NA replication and gained its selective advantage. It is proposed that the 'capped molecules', with the single amino acid 'caps' formed by the tNA, favored NA replication by directly promoting template-directed bond formation. An indirect enhancement of NA replication, e.g. by favoring the formation of particular bases or sugars, is considered unlikely because of the condition of partial isolation (where small molecules can diffuse away from the tNA).

Presumably the base sequence of the tNA resulted in a three-dimensional configuration with a catalytic site in which the spatial arrangement of bases, sugars, and

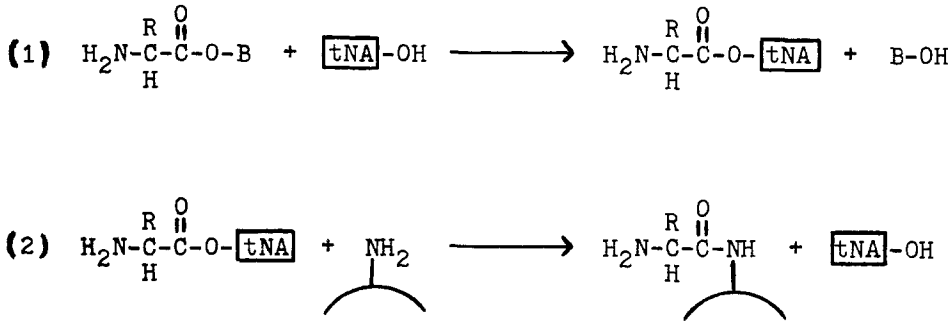

Fig. 3. The transfer NA (tNA) catalyzes the transfer of an activated amino acid to an accessible amino group in its environment. The transfer consists of two separate reactions: (1) The first reaction generates an 'activated tNA' [Both Woese (1967) and Smithies (quoted in Crick, 1968) have proposed that primitive tRNAs originally acted as their own activating enzymes]; (2) In the second reaction, the amino acid is transferred from the tNA to an accessible amino group, e.g. an amino group which is attached to the wall of the partial isolation chamber of the tNA. Note that both of these reactions are classified as transfer reactions, and are thus thermodynamically compatible with an aqueous environment. H_2N-(CHR)-(C = O)-O-B is an activated amino acid; B-OH is, for example, a phosphate.

phosphates greatly favored the transfer reactions shown in Figure 3. A tNA could use the α-amino group of activated amino acids as a 'handle' to attract and help hold an activated amino acid in the attachment site, thereby favoring the activation of the tNA. It is a particularly good handle for two reasons: (1) it carries a full positive charge, and (2) it is a fixed short distance from the activated carboxyl group of the amino acid. Although the *R* group of the amino acid does not directly participate in the transfer reaction, it is reasonable to suppose that the tNA would show considerable selectivity for which amino acids it would transfer because of the great differences in size, charge, hydrophobicity, etc. in the *R* groups of the various amino acids and the consequently widely varying abilities of the *R* groups to 'fit well' into the catalytic site of the tNA (Saxinger *et al.*, 1971).

How specific was the tNA for various amino acids? And how specific was the tNA for what accessible amino groups it would transfer its amino acid to? The possible answers to both of these questions range from 'highly specific' to 'relatively non-specific'. In both cases, an intermediate degree of specificity is considered most likely; however, the author finds no justification for ruling out any of the possibilities because all of them could plausibly the compatible with the only constraint on the resulting capped molecules (that their net effect is to significantly favor NA replication).

Although the amino acid attachment site of modern tRNAs is located at a terminal, the proposal that the original amino acid attachment site was located at a terminal is not favored because it is considered unlikely that environmental NA replication would have replicated terminals accurately – one or two bases or even long sequences could often be added at terminals as a result of replication. For this reason it is proposed that the structure and function of the tNA were compatible with unrelated sequences of varying lengths at both terminal ends of the tNA; and the

hypothesis is favored that an internal hydroxyl group (not associated with a terminal) functioned as the original amino acid attachment site.

As mentioned earlier, the tNA is proposed to have been somewhat specific for what amino acids it would transfer from the very outset. Since (1) it is likely that capped molecules with single amino acid caps consisting of the various amino acids would have widely varying influences on NA replication, and (2) specialization of the tNA to transfer a particular amino acid or group of closely related amino acids would facilitate the evolution of greater catalytic efficiency, it is proposed that the tNA evolved towards greater specificity for a particular amino acid or a group of closely related amino acids.

In summary, during this period, tNAs evolved as independent entities, each of which gained its selective advantage by altering the isolation chamber in which it was trapped. It did so by catalyzing the transfer of activated amino acids in solution to accessible amino groups in its isolation chamber as shown in Figure 3. This alteration of the tNA's microenvironment resulted in a local increase in the rate of environmental NA replication, and hence the selective advantage of the tNA.

6. Mainstream Evolution II: tNA-mNA

The proposed original tNA, which acted independent of other NAs, formed single amino acid caps on accessible amino groups. The ability to form 'dipeptide caps' would give an evolving entity a considerably greater potential influence on the molecules in its retention set, enabling it to form capped molecules with significantly greater abilities to favor NA replication. The formation of a dipeptide cap requires the formation of the following two bonds: the peptide bond between the two amino acids, and the bond linking the dipeptide to an accessible amino group. There are two fundamentally different ways for a pair of tNAs to form a dipeptide cap, which differ in the order in which these two bonds are formed. The two methods are described in the following two paragraphs; and in the subsequent paragraph, an argument in favor of the second method is presented.

Method 1: After one tNA had formed a single amino acid cap (attached to some previously accessible amino group), the second tNA could transfer its bound amino acid to that single amino acid cap, thereby forming a dipeptide cap. With this method, the bond linking the two amino acids is the second bond formed, and the tNA which forms the bond is reacting with a single amino acid cap. Thus the two tNAs do not come into contact with each other (a single tNA could form dipeptide caps in this manner). Note that this is the simplest case of a peptide synthesis apparatus which elongates the N-terminal of a growing peptide.

Method 2 (see Figure 4): One activated tNA could transfer its bound amino acid to the amino acid which is attached to the second tNA, thereby forming an activated dipeptide (bound to a tNA) which can be subsequently transferred to an accessible amino group. This second method involves the two activated tNAs reacting directly with each other. It is the simplest case of a peptide synthesis apparatus which elon-

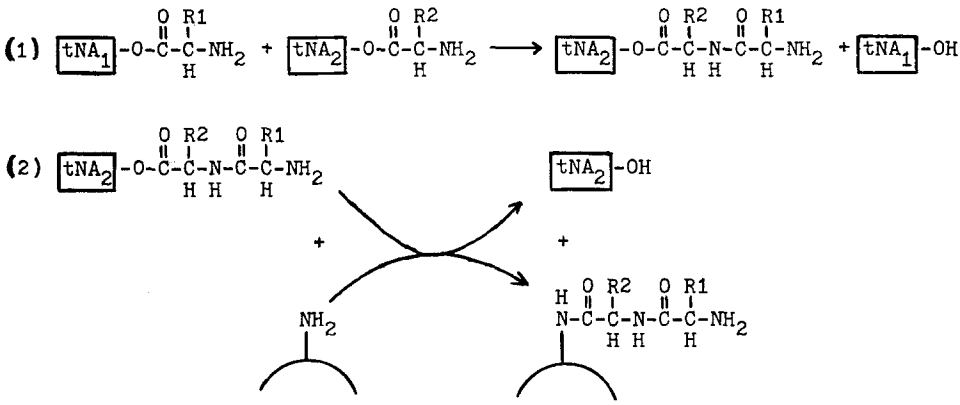

Fig. 4. Two tNAs jointly produce a dipeptide cap. (1) In the first reaction, the first activated tNA transfers its amino acid to the amino group of the amino acid which is attached to the second tNA, thereby generating an 'activated dipeptide'. (2) In the second reaction, the activated dipeptide is transferred to an accessible amino group in the environment (e.g. an amino group which is attached to the wall of the partial isolation chamber of the tNAs) thereby producing a 'dipeptide cap'. Note that the second tNA with the activated dipeptide could similarly have transferred its dipeptide to a third activated tNA, thereby producing an activated tripeptide.

gates the *C*-terminal of a growing peptide, and is further analogous to the modern peptide synthesis apparatus in that the growing peptide is transferred successively to the amino group of amino acids bound to tNAs.

The second method has an important advantage relative to the first: In the first method, the elongation of the cap is done while the cap is attached to some accessible amino group (not a tNA). In the second method, the elongation reaction takes place by the transfer of the amino acid (or growing peptide) to an amino acid which is still attached to the tNA which activated it, thereby allowing the tNA which is transferring the amino acid (or growing peptide) to recognize not only the amino acid to be transferred to but also the tNA which binds it. Because of this advantage and because the second method is analogous to that of the modern peptide synthesis apparatus, it is proposed that the second method (shown in Figure 4) was the one adopted by evolving entities in mainstream evolution.

The recognition of one tNA by another could take place by some means of binding, either direct (e.g. hybridization or steric interactions) or indirect (e.g. both tNAs hybridizing to a third NA) or a combination of the two. The evolution of this recognition and binding will be discussed later in this section.

There are several very different groups of amino acids represented in the modern genetic code (e.g. basic, acidic, bulky nonpolar, etc.) as well as some amino acids which fit in a class by themselves (e.g. glycine). In the preceding section ('mainstream evolution I'), it was proposed that the tNA (which was producing single amino acid caps) evolved towards specificity for a particular amino acid or a group of closely related amino acids. It is reasonable to suppose that some amino acids which were not significantly advantageous as single amino acid caps would be significantly advantageous as one of two amino acids of a dipeptide cap. Thus pairs of tNAs with

markedly different sets of amino acid preferences could co-evolve. At first the only new sets of amino acid preferences to evolve would be those which formed advantageous dipeptide caps together with the predominant set of amino acid preferences that evolved during the period of independently evolving tNAs; but as new sets of amino acid preferences became established, additional new sets of amino acid preferences could similarly have selective advantages.

There are two very different ways in which tNAs with markedly different sets of amino acid preferences could appear: (1) by mutation of a previous tNA; and (2) by a spontaneous creation of a new tNA by the environment. It is proposed that the first way (mutation) is what occurred because of the common characteristics of (nearly) all amino acids (e.g. the α-amino group and the α-hydrogen) which any tNA would recognize, and which might still be recognized (presumably somewhat less efficiently) by mutant tNAs with altered sets of amino acid preferences.

As mentioned earlier, recognition and binding of one tNA to another could be direct, indirect, or a combination of the two. In the very early stages of interactions, direct binding may have been the predominant means of binding since other means of binding would presumably require the successful replication of additional NAs; however, as 'speciation' of tNAs to activate different groups of amino acids takes place, there is a great advantage to a versatile system of ordering amino acids in caps so that tNAs can participate in the synthesis of several specific caps. One ordering system with a limitless ability to order tNAs is hybridization of the tNAs to another NA (a 'messenger NA' (mNA)). Note that a tNA-mNA system does not necessarily involve only the hybridization of the tNAs to the mNA, but could also involve a relatively weak interaction between adjacent tNAs along a mNA. Thus the relatively strong hybridization of the tNAs to the mNA could specify the ordering of the tNAs while the direct interactions of adjacent tNAs along the mNA could result in a positioning of the tNAs relative to each other such that the peptidyl transfer reaction is favored.

When two tNAs bound to a mNA form a dipeptide cap, translational peptide synthesis has occurred: information in the sequence of the mNA has been 'translated' into the dipeptide cap. The mNA, by binding those two particular tNAs, has determined the two sets of amino acid preferences which are represented in the two amino acids of the resulting dipeptide cap. Since the tNA-mNA system is the proposed original translational peptide synthesis apparatus, it is worthwhile to examine it closely: it is no more than a mNA holding two tNAs. The anticodons (called 'anticodons' because they decode the mNA) of the tNAs are most likely of different lengths; and the 'codons' of the mNA might not be perfectly complementary to the corresponding anticodons – rather the codons are determined functionally; by whether or not the anticodons will bind. The codons are most likely not adjacent, but rather are probably separated by a small number of bases.

With the advent of evolving NA communities, 'genomic NAs' – NAs which code for more than one (ideally all) of the members of a NA community – will aid the community in remaining intact through periods of redistribution, and could thus be selected for.

7. Mainstream Evolution III: tNA-mNA-rNA

The proposed tNA-mNA system could effectively produce relatively short peptide caps; however, a number of problems arise in production of longer peptide caps (and complete peptides) which are difficult to overcome with only tNAs and mNAs. Specifically, it would be advantageous for the evolving peptide synthesis apparatus to help ensure the following: (1) that translation is linear; (2) that the peptidyl transfer reaction occurs in the proper direction; (3) that initiation occurs at the right codon; (4) that only mNAs are translated; (5) that some mNAs are translated at higher frequencies than others; (6) that the terminal transfer reaction (or termination later in evolution) does not occur prematurely; (7) that only activated tNAs are able to bind adjacent to tNAs which are ready to transfer the growing peptide; and (8) that certain mNAs are translated under certain special conditions (translational regulation). Note that these improvements in the translation apparatus need not occur simultaneously; nor must they occur in any particular sequence; they could be acquired gradually one at a time. It is proposed that these improvements (and improvements which would simply favor the peptidyl transfer reaction) occurred by means of incorporation of additional NAs called 'ribosomal NAs' (rNAs) into the translation apparatus. Note that successive improvements in the translation apparatus need not always involve additional rNAs, but could also occur via alterations in existing rNAs to perform the additional functions or, especially later in evolution, via incorporation of biologically-synthesized peptides into the translation apparatus.

When the rNAs first interacted with the tNA-mNA system, they probably had to be compatible with a substantial amount of variability in tNA structure. However, the rNAs would function more efficiently with some tNA structures than with others, and thus would favor standardization of tNA structure. More importantly however, standardization of tNA structure and of the entire process of peptide synthesis would be favored *per se* (i.e. even arbitrary standardization) simply because it would allow the rNAs to specialize and improve a single standard peptidyl transfer reaction rather than attempting to evolve many varied peptidyl transfer reactions to and from tNAs of varying shapes and sizes. Note that the potential for standardization of tNA structure would be severely restricted while the tNAs still performed the catalytic activity of recognizing and activating amino acids. However, as the tNA-mNA and tNA-mNA-rNA systems evolved, this activity would gradually be taken over by activating enzymes produced by the systems and thus the structure of the tNAs would be able to standardize even further.

Thus, in summary, it has been proposed that the ability to synthesize polypeptides evolved gradually in small steps – one peptide bond at a time. The original tNA was able to form a single peptide bond and thus a single amino acid cap (Figure 3). Later, two tNAs jointly form two peptide bonds and thus a dipeptide cap (Figure 4); three tNAs jointly form the three peptide bonds in a tripeptide cap, etc. The introduction of mNAs brings a versatile ability to order specific tNAs for the formation of peptide caps with specific amino acid sequences. The introduction of rNAs brings greater efficiency and reliability, together with standardization.

8. Cell Wall

Earlier it was proposed that the 'cell wall' or (partial) isolation chamber of the earliest evolving NAs and NA communities was provided by the 'thick loosely-packed layer of tangled organic polymers' (see 'The environmental analogues of the cell wall and cell division'). The question of how an early isolation chamber could 'grow' (expand in volume due to an increased number of internal components) depends heavily upon the nature of the layer matrix. Although it is possible that the matrix was extensively covalently cross-linked such that isolation chambers were fairly rigid and nonexpandable, it is proposed that this was not the case, i.e., that noncovalent bonds played a large role in the structure of the matrix, and that therefore a growing NA community could often enlarge its isolation chamber by deforming the matrix.

Consider the optimal pore size of an isolation chamber which contains a given multicomponent NA system: (1) it would effectively retain the unattached NA(s) and unattached peptides (if any) of the system; (2) it would prevent large NAs from the outside from entering; and (3) it would allow a maximum number of the smaller substrate molecules (smaller NAs and activated amino acids) to diffuse in and out. Since the isolation chambers found in the environment would not be optimal (e.g. some pores could be larger than optimal), evolving entities would have a selective advantage if they could influence the porosity of their partial isolation chamber towards optimality.

It is proposed that the first influence of the entities on their chambers was to reduce the effective size of those pores which were larger than optimal. A lone tNA could reduce the diffusion of presumably highly negatively-charged NAs through pores by transferring amino acids with negatively-charged *R* groups to accessible amino groups of the chamber wall. Once the entities are capable of producing caps with several amino acids, the possibility of producing cross-links arises. The production of covalent cross-links could be catalyzed by enzyme-like caps; and noncovalent cross-links could be formed by pairs of caps which bind to each other. Note that these noncovalent crosslinks have the advantage of allowing increases in the size of the chamber due to the ability of individual cross-links to break once they are under a certain amount of tension. This could allow a very successful NA community within a given partial isolation chamber to grow in size for awhile before bursting the chamber; and, assuming the previous isolation chamber was in the interior of the thick organic layer, such a bursting of the chamber would simply release some of the NAs into a neighboring chamber where, in turn, they could replicate according to their ability. In this way, successful NA communities could spread through the layer.

Up to this point, the growing NA communities have acquired additional cell wall by expanding and deforming the organic polymers of the layer matrix. Real ability to synthesize cell wall must await the ability to synthesize a cell wall protein. A significant question is how such a cell wall protein would be held in place in the cell wall. Covalent cross-links are one possibility. However, there is the considerable problem of the mechanism whereby the cell wall protein would cross-link into a planar layer

rather than a three-dimensional lump. An attractive alternative is a cell wall protein which, due to its hydrophobic and hydrophilic surfaces, tends to assemble into a planar layer. Such a protein would tend to form a monolayer with the two hydrophilic surfaces facing the inside and the outside of the cell and with the hydrophobic band within the wall. Cell wall protein may have originally functioned by helping patch large holes in the environmentally-provided isolation chamber. Once a NA community was capable of synthesizing cell wall protein at a rate sufficient to keep pace with cell growth, it has the potential to become independent of the layer matrix.

Thus it is proposed that the evolving NA communities gradually evolved the ability to synthesize and repair a peptide-based cell wall.

9. Cell Division

Earlier it was proposed that the analogue of cell division in the early stages of evolution was the redistribution phase of the environmental retention and redistribution process. The ability to perform cell division has the important selective advantage of immediately reisolating progeny NAs (avoiding the possibility of being released into a very large body of soup); also cell division distributes the capped molecules (or complete peptides) of the previous isolation chamber among the daughter cells, thus giving the NAs of the daughter cells a favorably altered environment from the beginning of the new isolation.

Given an organic cell wall, cell division would be expected to occur at a low frequency via environmentally-caused movements of the cell wall. Once the evolving NA systems evolved the ability to patch holes in the cell wall with cross-links and cell wall protein (see previous section), the division event would not have to be entirely environmental. If the environment brought about a constriction of a cell (or isolation chamber) into two large compartments joined by a sufficiently narrow opening in the wall in common, the remainder of the division event could be performed biologically by the formation of cross-links and/or insertion of cell wall protein. A sophisticated improvement in the ability to patch holes (and to complete cell division events) would be the ability of some cross-links to contract – thus if a hole was too large to be spanned by cross-links or cell wall protein, one or more contracting cross-links along the circumference of the hole could reduce the size of the hole until it could be spanned. As the ability to patch holes evolved, cell division would become more frequent and more reliable since a greater range of environmentally-caused constrictions could initiate the division event, and thus the cell division process would gradually become less dependent on the environment and (more dependent on biological processes). Eventually the cell would evolve the ability to initiate cell division and control its timing and location.

Since even a single cell division event would have the selective advantages described above, biological cell division processes could evolve gradually, with cell division events gradually increasing in frequency from rare isolated events to increasingly long series of cell division events, until finally a very long series was initiated (which

continues today). Thus it is proposed that the redistribution phase of the environmental retention and redistribution process was gradually replaced by cell division as the means of reisolating the progeny NAs of successful NA systems.

Note that the exchange of hereditary information (sex) between different NA communities was easily accomplished by the redistribution phase of the environmental retention and redistribution process; however, with the advent of reliable cell division, specific mechanisms to allow exchange of hereditary information would have to evolve.

10. The Genetic Code

The genetic code originated with the first pair of tNAs capable of translating a mNA: it consisted of the two base sequences of the anticodons of the tNAs matched respectively with the two presumably different sets of amino acid preferences of the two tNAs. These anticodons were most likely much longer than the modern triplet anticodon, and the codons on the first mNAs were probably usually separated by small numbers of unassigned bases. Each new mutant tNA (which was a new match of anticodon sequence and set of amino acid preferences would mean an addition to the code; and the exact code in operation would depend on location – on which tNAs were present.

The proposed evolution of independent tNAs (see 'Mainstream evolution I') gave rise to a tNA which transferred a particular amino acid or a group of closely related amino acids. As evolving entities began to produce dipeptide (and longer) caps, there was a selective advantage for tNAs with completely new sets of amino acid preferences (see 'Mainstream evolution II'). Each of these new classes of amino acids represented in the code would presumably have its own distinct codon(s) which were probably significantly longer than the present triplet codon. During the initial part of this 'espansionary' phase, only a small minority of the set of possible codons would be represented in the genetic code. This situation has the potential advantage that random NAs which enter the system will tend to be ignored (rather than translated). However, a partially-filled genetic code has the disadvantage that most or nearly all mutations of the mNA will be nonsense mutations. Sonneborn (1965) and Crick (1968) have argued that too many nonsense codons would be selected against, so that most codons would 'quickly' be brought into use. This means that a numerous set of additional tNAs must be 'quickly' generated and maintained – adding a tremendous genetic burden onto the early evolving system. The genetic burden of maintaining the initially moderate number of mNAs would be far less. It is important to realize that the situation which Crick and Sonneborn are trying to avoid is nothing more than normal for evolving entities: the great majority of mutations will always be harmful.* An evolving entity could get to the stage of having a partially-filled genetic code only via its ability to reproduce all of its key sequences without lethal errors. From this

* Also, note that nonsense mutations may have only resulted in the deletion of the previously encoded amino acid since the proposed primitive translation apparatus was compatible with unassigned bases between codons.

relatively stable foundation, only those tNAs which conferred a substantial advantage to the system would be incorporated. Thus it is proposed that the code went through an expansionary phase during which the widely varying classes of amino acids which are represented in the modern genetic code came to be encoded.

It is proposed that the final evolution of codon assignments was a process of codon refinement (Woese, 1970) in which codons which originally code for groups of related amino acids gradually narrow their specificity until finally they code for single amino acids. Similarly, it is proposed that the present triplet codon length evolved as a result of codon length refinement (restricting the range of allowable codon lengths). With the original tNA-mNA system, anticodons of varying lengths were allowed. However, as the evolution of the tNA-mNA-rNA system brought about a standardization of tNA structure (see 'Mainstream evolution III'), it is proposed that there was a progressive restriction of the range of allowable codon lengths to eventually a triplet code, together with a gradual reduction and elimination of the allowableness of unassigned bases between codons.

There is undeniable order in the genetic code and there has been considerable discussion concerning the cause of that order (Crick, 1967, 1968; Dunnill, 1966; Goldberg and Wittes, 1966; Jukes, 1966; Sonneborn, 1965; Weber and Lacey, 1978; Jungck, 1978; Woese, 1965, 1967, 1969, 1973; Woese *et al.*, 1966; Hopfield, 1978). Woese has been the main proponent of the theory that there is a stereochemical relationship between the anticodons and their respective amino acids. However as Crick (1968) has pointed out, the absence of a stereochemical relationship would not imply that the order in the code is accidental. Regarding this paper, it is plausible that the base sequence of the early anticodons and the base sequence of the catalytic site of the early tNAs (whose bases would presumably have a stereochemical relationship with the amino acid(s) which they recognize and transfer) never overlapped – that the two sequences originally became linked by chance, and that subsequently throughout the evolution of the genetic code they performed their respective functions independently. This would mean that the first assignment of anticodon to set of amino acid preferences was random, and that the order in the code would have been generated by the mechanics of the expansion and standardization of the code. In turn, it is also plausible that originally there was an overlap between the sequences of the catalytic site and the anticodon, which could have resulted in a stereochemical relationship which could be recorded in the modern genetic code.

11. Discussion

Previous authors have made proposals which overlap the proposals of this paper. The proposal that the peptide synthesis apparatus was initially entirely NA has been made before (Woese, 1967; Orgel, 1968; Crick, 1968). Woese (1967, 1970, 1972), Orgel (1968), and Crick *et al.* (1976) have proposed models in which the primitive translation apparatus consists of mRNA and a few primitive tRNAs. Furthermore, Woese (1967, 1970, 1972) has proposed a period of pretranslational evolution which pre-

ceded and contributed to the evolution of the translation apparatus. However, although the outlines of these proposals overlap that of this paper, the entities proposed by these authors are completely different in many profoundly significant ways from the entities of this paper.

The proposed original pretranslational entity of this paper, the original tNA, has a single catalytic site with the ability to transfer activated amino acids to accessible amino groups. Via this ability it forms a single bond per capped molecule (and capped molecules in turn favor NA replication). In contrast, Woese (1967) proposes a 'proto-tRNA' with two sites which carries out initiation and elongation, thus creating a 'copeptide of related amino acids'. In the following stage, two such proto-tRNAs together produce mixed polypeptides, and subsequently the interaction of the two proto-tRNAs is made dependent upon a third RNA, the prototype of mRNA. Note that Woese's proposed proto-tRNA has two sites, and that it forms entire peptides in which every peptide bond has been formed by the apparatus.

The proposed original translation apparatus of this paper, the original tNA-mNA system, is no more than two tNAs bound to a third NA (the mNA) by means of hybridization. The mNA thereby promotes the formation of dipeptide caps. The genetic code which comes into existence with this tNA-mNA system is compatible with varying anticodon lengths and unassigned bases between codons. In the course of evolution, the proposed translation apparatus gradually evolves the ability to produce longer and longer peptide caps, thereby gradually evolving the ability to synthesize complete peptides. In contrast, Crick *et al.* 's model calls for an overlapping quintuplet code which imposes considerable order on mRNAs and tRNAs, and Woese's and Orgel's primitive tRNA-mRNA systems use a triplet code. In all of these cases, the proposed primitive systems synthesize entire peptides in which every peptide bond has been formed by the apparatus.

The key to the origin of life is that initially the essential processes (NA replication, energy supply (supply of activated compounds), cell wall (retention), and cell division (redistribution)) were carried on (albeit very inefficiently) by the environment, and subsequently, with this essential supportive environment, the evolving entities gradually increased their ability to favorably influence the environmental processes until finally the entire processes were carried on biologically.

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