J Mol Evol (1989) 29:465–474

Journal of
Molecular Evolution
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The Origin of Polynucleotide-Directed Protein Synthesis

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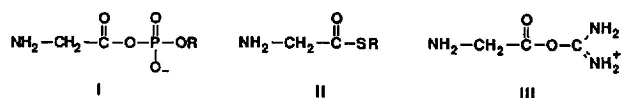
Summary. If protein synthesis evolved in an RNA world it was probably preceded by simpler processes by means of which interaction with amino acids conferred selective advantage on replicating RNA molecules. It is suggested that, at first, the simple attachment of amino acids to the 2′(3′)-termini of RNA templates favored initiation of replication at the end of the template rather than at internal positions. The second stage in the evolution of protein synthesis would probably have been the association of pairs of charged RNA adaptors in such a way as to favor noncoded formation of peptides. Only after this process had become efficient could coded synthesis have begun.

Key words: Prebiotic peptide synthesis — RNA world — Activated amino acid — Aminoacyl adenylate

Introduction

Protein synthesis is a complex process that involves some scores of proteins and polynucleotides. Clearly, it must have evolved through a series of simpler stages. In this article the relevant nonenzymatic, aqueous solution chemistry of amino acids is first discussed, followed by a discussion of attempts to construct plausible evolutionary sequences leading from noncoded, peptide-forming reactions to protein synthesis. Many of the arguments presented here have previously been published by the present author and by others. I do not attempt to review the extensive literature on prebiotic peptide synthesis (Fox 1978), except insofar as it is relevant to the evolution of polynucleotide-coded synthesis.

The synthesis of peptides from amino acids in an aqueous environment must involve the preliminary activation of the carboxyl group of the amino acid. The nature of the activation process on the primitive earth is unknown. A number of primary activating reactions have been suggested (Hulshof and Ponnamperuma 1976). These include reaction of the amino acid with a molecule containing a pyrophosphate bond to yield a phosphate anhydride (I), formation of a thioester (II), and reaction with a condensing agent such as cyanamide or its dimer to yield a reactive, high-energy intermediate (III).



The carboxyl group of an activated amino acid in an aqueous environment will ultimately be transferred to one or another of the nucleophiles present in the solution. If the amino acid is present at a very low concentration, and if no other nucleophiles are present, the activated derivative will undergo hydrolysis by water to regenerate the original amino acid (Fig. 1).

In the absence of extraneous nucleophiles, if the concentration of the amino acid is high enough, peptide bond formation will compete with hydrolysis (Fig. 1). The nature of the products formed will depend somewhat on the rate of activation. If an activating agent is introduced slowly into a solution of the amino acid, only a small proportion of the amino acid (or peptide) is activated at any time. Then the predominant first product is always the nonactivated dipeptide. Subsequently, higher peptides are formed by the consecutive addition of ac-

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$$NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}\sim X \xrightarrow{H_2O} NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OH + XH$$

$$NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}\sim X \xrightarrow{NH_2{-}CH_2{-}COOH} NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}NH{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OH + XH$$

Fig. 1. The reactions of an activated amino acid in aqueous solution.

tivated amino acids to the dipeptide. However, activation of the dipeptide must occur, to some extent, in parallel with activation of the monomer, and will generally lead to the formation of a diketopiperazine, i.e., a nonreactive, cyclic dipeptide (Fig. 2). Once a dipeptide has been extended beyond the dimer, to the trimer or to a higher oligomer, cyclization is very much less efficient.

Batch polymerizations of pure or nearly pure activated amino acids proceed in a somewhat different way. The first product, formed in large amount, is the activated dimer which, as we have seen, often cyclizes efficiently to yield the diketopiperazine (Fig. 3). The yield of longer oligomer, which depends on the relative rates of elongation and cyclization of the activated dimer, may be small.

At this point one must mention a special case that may be important. In the presence of carbon dioxide or the bicarbonate ion, an amino acid is partially converted to a carbamate. Activation of a carbamate usually leads to the formation of a cyclic anhydride, the Leuchs anhydride (Brack 1984) (Fig. 4). Leuchs anhydrides polymerize efficiently in aqueous solution by a mechanism that does not lead to the formation of diketopiperazines.

$$NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}NH{-}CH_2{-}\overset{O}{\overset{\|}{C}}\sim X \longrightarrow \text{diketopiperazine} + XH$$

Fig. 2. The formation of a diketopiperazine from an activated dipeptide.

We must next consider the effect of an additional nucleophile on the system. In general, any nucleophile will compete for the activated intermediate with water and with the amino acid itself. In many cases, e.g., if the nucleophile is an imidazole or a nucleoside, the result is the formation of a secondary activated intermediate. Because secondary activated intermediates may have very different chemical properties from the primary activated intermediate, a great deal of flexibility is introduced into the chemistry of activated amino acids. In nature, this has made possible the evolution of coded protein synthesis. We cannot discuss all of the different possibilities in detail. Here we consider nucleosides and their derivatives as secondary nucleophiles, as this is the case that is directly relevant to the origin of protein synthesis.

The Implausibility of Unevolved Polynucleotide Adaptors

In the course of protein synthesis, amino acids are activated by ATP via aminoacyladenylate anhydrides and transferred to adaptors, tRNA molecules, prior to the formation of peptide bonds. In this section I will argue that the synthesis of peptides by an analogous sequence of reactions would not occur significantly in the absence of enzymes or of complex RNA molecules that had already evolved to carry out efficiently some of the reactions that are nowadays part of coded peptide synthesis.

$$NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}\sim X + NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}\sim X \longrightarrow NH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}NH{-}CH_2{-}\overset{O}{\overset{\|}{C}}\sim X + XH$$

$$\longrightarrow \text{diketopiperazine} + XH$$

Fig. 3. The formation of diketopiperazines in the batch-polymerization of an activated amino acid may prevent the formation of oligopeptides.

Fig. 4. Free amino acids react with CO_2 to give carbamates. The activation of carbamates leads to the formation of the cyclic Leuchs anhydrides. These anhydrides polymerize spontaneously in aqueous solution to give polypeptides, often with little or no diketopiperazine formation.

The hydrolysis and aminolysis of aminoacylesters of nucleosides, nucleotides, and polynucleotides have been studied in some detail on account of the relevance of these reactions to the hydrolysis of aminoacyl tRNA and the formation of peptide bonds on the ribosome (Zachau and Feldmann 1965; Hentzen et al. 1972; Schuber and Pinck 1974a,b,c; Lacey and Mullins 1983). Similar studies, motivated by an interest in prebiotic chemistry, are less numerous, although in some cases they are more closely related to the problem of the origin of coded protein synthesis (Weber and Orgel 1978a,b, 1979).

The most important reactions that are to be anticipated when an amino acid is activated in the presence of a nucleotide or oligonucleotide are illustrated in Fig. 5. We must ask how far the adaptor-dependent reaction scheme (arrows drawn heavily in the figure) can compete with syntheses of peptides that occur by any of the pathways that involve one or two nonesterified amino acids as reactants.

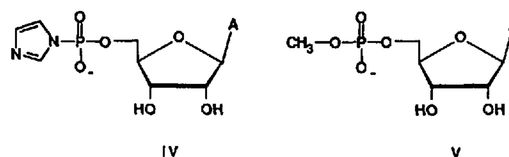
In order for adaptor-dependent synthesis to compete effectively with more direct synthesis, two conditions must be satisfied. First, reaction between the activated amino acid and the adaptor must compete effectively with direct reaction between the activated amino acid and a free amino acid. Second, the reaction between two charged polynucleotide adaptors must compete effectively with the reaction be-

Fig. 5. The reactions of activated amino acids in the presence of oligonucleotides. The 3′ terminus of an oligonucleotide is designated as a cis-glycol. The adaptor-dependent route is emphasized by the use of heavily drawn arrows.

tween a charged adaptor and a free amino acid. Whether or not these conditions can be met will depend on the kinetics of the various synthetic and hydrolytic reactions shown in Fig. 5 and on the rate at which activated amino acids are produced in the system.

The reaction of activated amino acids with oligonucleotides, the hydrolysis of the aminoacyl esters, and all of the other relevant reactions have rates that depend strongly on the identity of the amino acid, the pH, temperature, salt concentration, etc. The many published experimental studies have not been carried out under a single, consistent set of conditions (Zachau and Feldmann 1965; Hentzen et al. 1972; Schuber and Pinck 1974a,b,c; Lacey and Mullins 1983). Fortunately, the results obtained in one set of studies specifically designed to explore the possibility of adaptor-dependent peptide synthesis under conditions chosen to favor this reaction as much as possible (Weber and Orgel 1978a,b, 1979) seem generally consistent with those reported by other authors.

The aminoacylimidazoles are particularly efficient reagents for the aminoacylation of alcohols and have been used extensively in the preparation of aminoacyl esters of nucleosides, nucleotides, and polynucleotides. Prebiotic activation was, therefore, modeled by incubating the amino acid with adenosine-5′-phosphoroimidazolide (IV) (ImpA), under conditions that lead, indirectly, to fairly efficient formation of the aminoacylimidazole derivative (Weber and Orgel 1978a). In these experiments the 3′-terminus of an oligonucleotide was replaced by the methyl ester of adenosine-5′-phosphate (V) (MepA) as an aminoacyl acceptor (Fig. 6). All concentrations were held at levels higher than could reasonably be anticipated under prebiotic conditions, namely 0.1 M ImpA and 0.5 M each of MepA and of the amino acid.



In these experiments the accumulation of MepA-ala ester at pH 5.8 was as large as 10.4%, based on the ImpA consumed. At pH 7, however, the accumulation was only about 2.5%. Even at pH 5.8, a pH that is unfavorable for the formation of peptides, the yield of glycylglycine from glycine and ImpA was about twice the yield of MepA-gly ester. A detailed analysis of these results suggests that, under conditions that are chosen to favor the formation of esters, the reaction of an aminoacylimidazolide with glycine is at least as fast as its reaction with a nucleoside. At pHs close to neutrality, or with amino acids activated in other ways, peptide formation would be substantially faster than the formation of the aminoacyl ester of the nucleotide.

The peptide bond-forming reaction of protein synthesis was modeled by studying the autoconden-

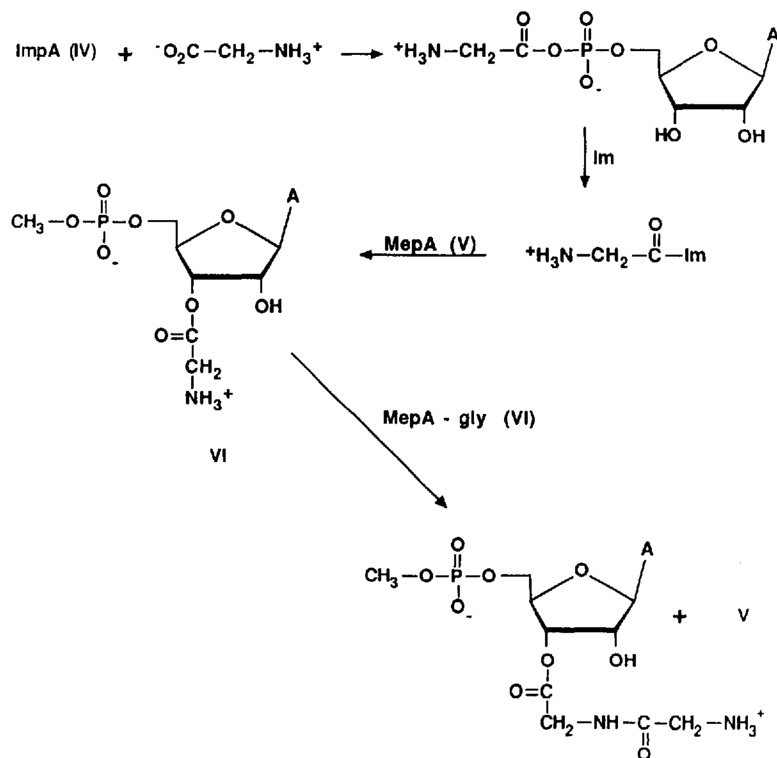


Fig. 6. The reactions involved in the synthesis of peptides from an amino acid and ImpA in the presence of MepA.

sation of MepA-gly ester in aqueous solution at various pHs (Weber and Orgel 1979). To force the reaction in the direction of peptide formation, a very high concentration of the pure ester, 0.43 M, was used. The most striking result of these experiments was the finding that larger oligopeptides cannot be detected. Under optimal conditions the yield of triglycine was less than 1%. The combined yield of diglycine and the cyclic dipeptide, diketopiperazine, never exceeded 20%.

The two experiments described above show that, even under very favorable conditions, the aminoacylation of a nucleotide and peptide bond formation by the reaction between aminoacyl esters of nucleotides are inefficient reactions. Although dipeptides and diketopiperazines are formed in significant amounts from aminoacyl esters of nucleotides, the yield of polypeptides is negligible. Under all plausible conditions the dipeptides formed more efficiently by the reaction of the free amino acid with activated amino acid or the aminoacyl ester of a nucleotide than by the reaction between two molecules of the aminoacyl ester. A more detailed discussion suggests that even if it were possible to achieve a 0.4 M concentration of the ester in the absence of free amino acid, the efficiency of condensation would still need to be increased several hundredfold to obtain substantial yields of polypeptides (Weber and Orgel 1979).

A number of amino acids, e.g., serine, condense more efficiently than glycine and alanine (Weber and Orgel 1978b; Weber 1987). It might also be possible to find conditions of temperature, pH, and ionic strength that would increase the efficiency of adaptor-mediated peptide synthesis somewhat. Nonetheless, the experiments show that no plausible prebiotic reaction scheme would be compatible with an efficient, polynucleotide-mediated synthesis of oligopeptides, unless the RNA adaptor had evolved properties very different from those to be expected of a random oligonucleotide (Weber and Orgel 1979).

There is another problem associated with the evolution of adaptor-directed peptide synthesis in a system containing naive oligonucleotides. Protein synthesis involves the attachment of amino acids exclusively to the 3′-terminus of tRNAs. However, the nonenzymatic reaction between an activated amino acid and a polynucleotide is much less specific. The rate of reaction at an internal 2′-hydroxyl group is very dependent on the sequence and structure of the RNA. It can occur at a rate only two or three times slower than at the terminus in short oligomers or at very low rates in double-stranded RNAs (Weber and Fox 1973; Weber and Lacey 1975; Profy and Usher 1984a,b; Usher and Needels 1984). It seems unlikely that end addition would be the dominant reaction, except in the case of RNAs that had very extensive self-structure.

The Evolution of Peptide Synthesis in an RNA World

The arguments given above suggest that adaptor-dependent polypeptide synthesis would not occur to a significant extent in a world of random polynucleotides. In the second part of this paper I will present far more speculative arguments concerning the evolution of protein synthesis in a preevolved RNA world. We accept, provisionally, the suggestion that, prior to the evolution of protein synthesis, there were organisms based on RNA as a genetic material that also used RNA catalysts (ribozymes) to facilitate replication and perhaps to support a more or less elaborate metabolism (Woese 1967; Crick 1968; Orgel 1968; Sharp 1985; Gilbert 1986; Lewin 1986; Benner et al. 1987). We propose that protein synthesis evolved in such an RNA world as an adaptation in which amino acids were utilized by RNA organisms to improve their fitness.

This model raises a difficulty of a kind that is very familiar in evolutionary theory. Many adaptations are so complex that it would be unreasonable to suppose that they arose in a single step. However, in many cases, intermediate stages in the evolution of the adaptation would not be at all useful in the context of its final form. When faced with such problems, it is usually assumed that the intermediate stages did provide a selective advantage, but one that had little or no relation to the function of the final adaptation.

The problem of the origin of protein synthesis should be approached in this way (Orgel 1972). Coded protein synthesis is so complex a process that there is no possibility that it could have appeared de novo in a family of RNA molecules that had evolved to carry out different and unrelated functions. A solution along conventional lines is, therefore, to suppose that coded protein synthesis is the consequence of a series of adaptations, each of which conferred selective advantage through some function simpler than coded protein synthesis. It is also possible that protein synthesis represents a relatively simple modification of some process that was already part of the metabolism of the RNA organism. I will discuss the first possibility in some detail, and make only a few remarks on the second.

The first step in the evolution of protein synthesis almost certainly was the appearance of RNA adaptors that captured amino acids from their environment. It seems very probable that the amino acids became attached to the 3′-termini of RNA molecules by ester bonds. Such bonds could most easily be formed by the attack of the 3′-termini of RNA molecules on activated amino acids by a mechanism similar to those discussed in the first part of this paper.

It has often been suggested that nucleotide co-

factors are fossils of the RNA world (Woese 1967; Orgel 1968; White 1976; Mar et al. 1987). It is argued that the presence of a nucleotide in such cofactors as nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) cannot be explained readily in terms of their specific functions; much simpler derivatives of nicotinamide, for example, have chemical properties very similar to the cofactor NAD. It is claimed that the only plausible explanation of the involvement of nucleotides is that the cofactors are relics of an RNA world in which catalysts were equipped with nucleotide handles to bind them by specific hydrogen bonds to polynucleotide carriers. Similar arguments suggest strongly that the nucleotide-containing aminoacyl adenylates, the substrates of modern protein synthesis, were also the activated amino acids of the RNA world. Another model has been described in which an unactivated amino acid displaces an oligonucleotide from the 3′-OH group of an internal nucleotide in a transesterification reaction that is closely related to splicing (Weiner and Maizels 1987).

Is it reasonable to postulate that amino acids could attach efficiently and specifically to RNAs that had evolved to accommodate them? One example of an RNA sequence capable of associating specifically with arginine has been described (Yarus 1988). There is no experimental evidence for RNAs that not only bind specifically but also react with activated amino acids to form ester bonds. However, the work of Usher and of Weber and Lacey cited above shows some degree of specificity in the acylation even of homo-oligonucleotides, so more complex RNAs with well-defined secondary structures might well react specifically with activated amino acids. (The adenosine handle in the aminoacyl adenylates would have greatly simplified the task of placing the amino acid in an environment that provides adequate specificity.) A priori, the site of interaction might equally well be any internal 2′-OH group of the RNA or the terminal cis-glycol group.

To explain the appearance in the RNA world of a family or families of oligonucleotides that specifically attached amino acids to their 3′-termini, we must show how such an attachment could have improved the fitness of the relevant RNAs. Several possibilities come to mind. The amino acid may have anchored the RNA in an advantageous environment, for example to the negatively charged surface of a mineral. Another possibility, which I find particularly appealing, is that the amino acid facilitated the initiation of replication at the 3′-terminus of the RNA.

One of the major side-reactions encountered in attempts to carry out template-directed replication is the initiation of synthesis at internal positions on the template (Acevedo and Orgel 1987). The net positive charge on an aminoacyl ester (or a peptidyl ester) might help to direct the first nucleotide to the 3′-terminus of the template, and so to guarantee the formation of full-length rather than partial copies. This, of course, would explain why RNAs evolved to fix amino acids specifically at their 3′-termini rather than at internal positions.

If the attachment of the amino acid to the 3′-end of an RNA conferred a selective advantage, for any reason, the RNA would have evolved a base sequence that favored efficient charging and increased resistance to hydrolysis of the adduct. The RNA would, of course, also have been constrained to perform its appropriate catalytic function in the RNA world. Thus, we might anticipate the evolution of one or more ribozymes, which carried amino acid-binding sequences at their 3′-termini. If the sequence constraints imposed by the requirement for catalytic activity were severe, the terminal amino acid-binding domain might have been independent of the ribozyme functional domains.

Next we must consider selection between amino acids. If all of the amino acids in the prebiotic environment provided equal selective advantage, there would be no reason for the evolution of selectivity among amino acids. However, if some amino acids formed adducts that initiated more rapidly than others or were more stable than others, we would expect RNAs to evolve to select just those amino acids. Thus, one might expect one or more ribozymes adapted to combine with one amino acid or a small group of structurally similar amino acids (Woese 1967). Different RNAs would probably have selected different amino acids.

The stable, probably selective, attachment of amino acids to the 3′-ends of tRNA molecules would be a step in the direction of protein synthesis. However, there is a priori no reason why the adducts should react preferentially with each other rather than with free amino acids in solution. To explain the evolution of efficient peptide formation, we must, therefore, propose that the formation of short peptides attached to RNAs was more advantageous than the attachment of single amino acids. One possible advantage might arise from the greater stability of peptide–oligonucleotide adducts. Peptidyl esters have lifetimes against hydrolysis that are an order of magnitude greater than those of aminoacyl esters (Gilbert 1963; Schuber and Pinck 1974a).

Provided the formation of peptides was advantageous, for whatever reason, natural selection would have favored the evolution of RNAs that associated together in such a way as to facilitate the formation of peptides from amino acids attached to their 3′-termini. The mechanism of association is not specified by this theory. It could have involved only Watson–Crick base-pairing, but it might also have depended, partially or entirely, on other interactions.

The development of a noncoded mechanism for the synthesis of short peptides attached to RNA introduces the most puzzling phase in the evolution of protein synthesis—the appearance of cooperativity between an external messenger RNA and the RNAs that facilitated peptide synthesis. I suggest that the process of peptide synthesis at the terminus of RNA first acquired many of the characteristics of protein synthesis other than coding. RNAs associated in such a way that a peptide attached to one RNA was transferred efficiently to an amino acid at the end of another. This led to the production of peptides that were longer than those produced by direct synthesis from activated amino acids. These longer peptides were partially ordered, as there was some specificity in the charging of RNAs by amino acids and in the association of RNAs with each other. Sooner or later families of peptides were formed that, when detached from the RNAs on which they were synthesized, conferred selective advantage on the RNA organism as a whole.

Once detached peptides became useful to an RNA organism, the stage was set for the separation of the amino acid binding domains from their carriers and the evolution of RNAs whose only function was to facilitate peptide synthesis. I call these RNAs adaptors—they were the ancestors of tRNAs. The evolution of protein synthesis, from now on, is largely the evolution of adaptors.

It seems probable that the first adaptor-synthesized peptides fulfilled a structural rather than a catalytic function in the RNA world (Brack and Orgel 1975; Jay and Gilbert 1987). I particularly like the scheme in which two adaptor RNAs, one preferentially carrying a hydrophobic and the other a hydrophilic amino acid, cooperated to produce alternating polypeptides. I have argued elsewhere that such peptides would have tended to form membrane-like structures that acted as "glue" to keep families of polynucleotides together (Orgel 1972; Brack and Orgel 1975).

The Evolution of Coding

The essence of coding is the influence of a polynucleotide messenger on the sequence in which amino acids are assembled. It is tempting to think of the modern genetic code as arising through a sequence of simpler codes that had fewer elements but which obeyed rules as rigorous as those that operate now. It has often been proposed that at first there was only a restricted set of amino acids and codons, and that later new amino acids were introduced and assigned to previously unused codons (see, e.g., Lehman and Jukes 1988). All such theories, although immensely attractive to theoreticians, assume a degree of chemical specificity at an early stage of evolution that seems to me implausible in light of our knowledge of noncovalent chemical interactions. I would like to propose that the evolution of the code was more like the coming into focus of a fuzzy image than the extension of a set of mathematical equations to ever larger numbers of variables.

Looked at from this point of view, one can ask what the very simplest form of coding would be like. Suppose one had two or more types of adaptors, each specialized to combine with a specific set of amino acids (the sets might overlap), and each with a characteristic affinity for itself and for other charged adaptors. The consequence would be the synthesis of an ensemble of peptide sequences, whose statistical distribution would depend only on the abundances and properties of the different charged adaptors and the abundance of activated amino acids. Polynucleotide-dependent coding, in its broadest sense, is the introduction of a new interaction by means of which external polynucleotides can influence the statistical distribution of products in a way that depends on the sequence of the external polynucleotide message. One might, for example, have one external polynucleotide that favored the appearance of alternating polypeptides, and another that favored the clustering of hydrophobic amino acids. The production of more than one statistical ensemble of polypeptides using only one set of adaptors is the *minimum requirement* for coding.

The nature of the interaction between the messenger and the adaptor RNAs is not indicated by the model. However, with the advantage of hindsight, we can see that the ultimately successful interaction must have involved base-pairing between exposed regions of the adaptor RNAs and contiguous triplets of the messenger. The optimization of this interaction led to the appearance of pre-tRNAs, i.e., RNA molecules that become charged more or less specifically with amino acids and that could be ordered on a messenger RNA to favor the production of specific families of polypeptide sequences. The regions of tRNAs that interact with proteins must have evolved later.

A striking feature of protein synthesis that is rarely emphasized is the use of *contiguous* base triplets in the message to code for contiguous amino acids. Our model provides no obvious reason why this should be true—the message might, for example, have been read three bases at a time by the adaptors, but a fixed number of bases between each pair of coding triplets might have been ignored. Considering the crowding that would be expected at the junction between a message and two pre-tRNAs that had not been specially designed for coded synthesis, the interpolation of one or two irrelevant bases between each pair of codons seems very likely. One could, of course, argue that it must be possible to line up two anticodons on a pair of contiguous mes-

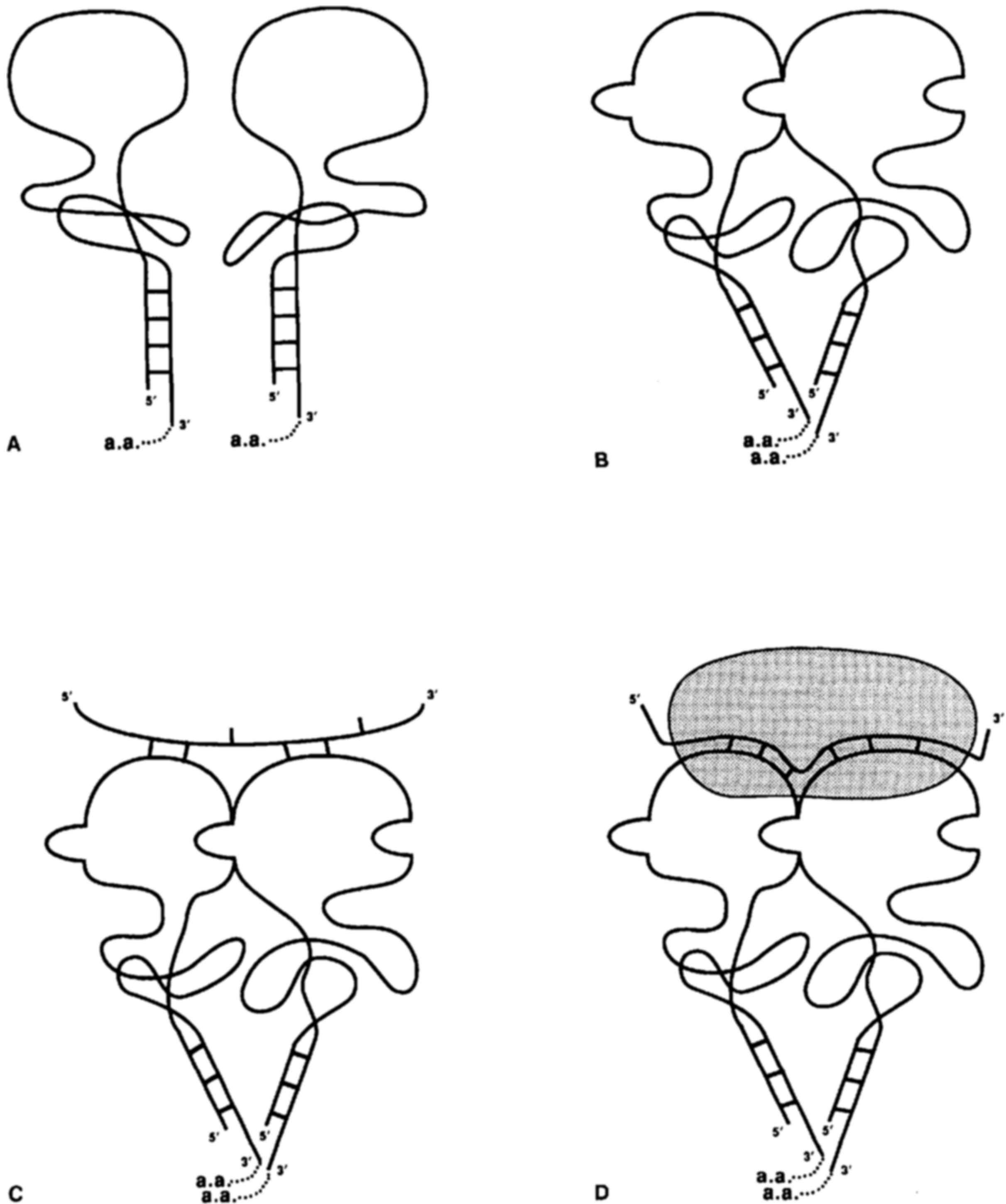


Fig. 7. Hypothetical stages in the evolution of protein synthesis. **A** The evolution of RNAs that attach amino acids to their 3'-termini. **B** The development of interactions between adaptor RNAs that favors peptide bond formation. The nature of the contacts between RNAs is not specified in the model. **C** Interaction between the adaptor RNAs and a message that enhances any preexisting specificity. At this stage, reading is two out of three. **D** Accurate, three out of three reading made possible by the ribosome.

senger triplets, and that this arrangement won out because it is the most efficient in compressing coding information into a message of fixed length. However, the present approach suggests the possibility of a different explanation.

It has been argued that the energy of interaction of complementary base triplets with each other is inadequate to permit the stable association of tRNAs with a messenger, and that as many as 5 bp were used early in the evolution of protein synthesis (Crick et al. 1976). It has also been emphasized that many aspects of protein synthesis proceed as though only 2 bp out of 3 matter (Lagerkvist 1978; Samuelsson et al. 1987). How can one resolve this apparent con-

flict? If tRNAs associated with each other to facilitate peptide synthesis even in the absence of a messenger, the codon–anticodon interaction would be needed only to supply additional specificity. Two base pairs would then have been adequate for this purpose. I suggest that the abstract "two out of three" character of modern protein synthesis reflects a structural "two out of three" reading in early coded peptide synthesis. Two bases provided specificity, but the message was traversed three bases at a time. The presence of one nonpaired base between paired doublets provided the flexibility needed to fit two pre-tRNAs onto the message. Only later, after the evolution of ribosomal proteins and of tRNA modifications, was use made of the third position (Fig. 7).

Many of the details that I have suggested above are arbitrary. The proposals that I consider central are

1) Polynucleotides first evolved to attach amino acids to their 3′-termini in as stable a way as possible because this was advantageous in a context unrelated to peptide synthesis.
2) Association between adaptor polynucleotides that led to the weakly specific synthesis of families of peptides evolved next, before messenger-directed synthesis.
3) The early stages in the evolution of the genetic code involved the gradual modification and extension of the preexisting, weakly sequence-specific, noncoded system of polypeptide synthesis.

Alternative Theories

Weiner and Maizels have discussed, in particular, the origin of the charging mechanism for RNA adaptors (Weiner and Maizels 1987). They suggest that the transesterification mechanism that is involved in the splicing of type I introns was already in place at the time that the evolution of protein synthesis began. A ribozyme that normally caused the attack of the 3′-OH group of one oligonucleotide on the sensitive phosphodiester bond of another came to accept an amino acid as an alternative substrate, thereby creating an aminoacylated adaptor RNA. The chemistry involved in their ingenious model is unorthodox, but perhaps no more unorthodox than the established chemistry of the splicing mechanism for type I introns (Cech 1987). Weiner and Maizel's theory (1987) is compatible with the remainder of the argument of the present paper.

If ribo-organisms had a sufficiently complex metabolism to be able to synthesize cofactors such as NAD and FAD, perhaps they also synthesized specific, catalytically active dipeptides or short polypeptides via adaptor RNA intermediates. This would have provided an entry into coded synthesis that was highly specific from the beginning. This model is more in line with many abstract theories of the origin of the genetic code. It seems to me less likely than the theory outlined above, but certainly possible.

Acknowledgments. This work was supported by grant NGR 05067001 from the National Aeronautics and Space Administration and grant GM-13435-21 from the National Institutes of Health. We thank Aubrey Hill, Jr., for drawing the figures, and Sylvia Bailey for manuscript preparation.

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Received April 14, 1989/Revised and accepted June 23, 1989