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Molecular Replication and the Origins of Life

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1. Introduction

All living organisms have a great deal in common. The interior environment of their cells is a concentrated aqueous solution containing a characteristic set of organic macromolecules. They are surrounded by membranes composed of a different set of organic molecules called lipids. This similarity in composition results from an even more striking similarity in organization. The universal genetic system of all forms of life is dependent on a very complicated interplay between two sets of macromolecules—proteins and nucleic acids. The proteins are made up by joining 20 standard amino acids together in specific linear sequences, while the nucleic acids are formed in a similar way from 4 standard nucleotides. Membranes are somewhat more variable, but all are constructed according to the same general plan. They consist, in the main, of an impermeable bilayer of lipid molecules in which specific protein-carrier molecules and channels are embedded.

It became clear very early in the history of chemistry that nothing at all like the components of living systems occurs on the earth in a non-biological context—hence, the traditional division of chemistry into organic and inorganic subdisciplines. The chemists' interest in the problem of the origins of life is mainly concerned with the origin of organic material on the primitive earth before the appearance of life and with the sequence of events that led to its self-organization into a living system.

In retrospect one can see that Wöhler's synthesis of urea from ammonium cyanate in 1828 [1] was an important step toward our understanding of the origins of life. Prior to this discovery it was thought to be impossible to produce organic products from inorganic starting materials without the assistance of a "vital force". Since urea is organic and ammonium cyanate can be produced from inorganic sources, the publication of Wöhler's results made it clear that organic material could originate in an inorganic environment through normal abiotic processes.

The investigation of reactions that produce important biochemicals from elementary constituents known to be present in planetary atmospheres was initiated by the speculations of Oparin [2] and the experimental studies done by Miller in Professor Harold Urey's laboratory [3]. This subject has been reviewed repeatedly [4]. The basic finding is that mixtures containing some or all of the elementary atmospheric constituents, hydrogen, water, nitrogen, ammonia, methane, carbon monoxide and carbon dioxide, when subjected to "high energy" sources such as ultraviolet irradiation or electric discharges, yield complex mixtures of products including amino acids, nucleotide bases and a variety of other biochemicals. These laboratory studies are complemented by the observation of a great variety of small organic molecules in the dust clouds where new stars form [5] and by the discovery of amino acids and other organic molecules as indigenous constituents of certain stony meteorites [6].

At the present time, the sources of the organic material that must have accumulated on the primitive earth before the emergence of life is obscure. There are too many possibilities. Synthesis in the atmosphere or in volcanoes or deep-sea vents is one possibility. Accumulation as constituents of the material from which the earth accreted, or from meteorites falling on the primitive earth, is another. It is, however, abundantly clear that organic material, including a surprising number of important contemporary biochemicals, is formed in the cosmos without the intervention of living organisms, intelligent or otherwise. The origin of the organic chemicals in the prebiotic environment is now a subject for detailed study, but does not present major conceptual difficulties.

Direct evidence concerning the steps leading from the mixture of organic compounds that accumulated on the primitive earth to an organized living system has not survived in the geological record. Thus we can turn only to laboratory experiments and to the properties of contemporary living organisms for relevant information. The obstacles to the formulation of a detailed and plausible model, given these limitations, are formidable. It is not surprising that there is at present no coherent, generally accepted model for the origins of life.

Attempts to recapitulate in the laboratory the actual events that occurred on the primitive earth are doomed to failure. We do not know how long it took for life to evolve once the conditions necessary for its appearance were present. Although the upper limit placed by the geological record is about one thousand million years, the evolution of life could have taken a very much shorter time. However, it is unlikely to have taken a time as short as a human lifetime. We are forced, therefore, to search for model systems which provide useful information in days about processes that may have taken millenia. Similarly, the scale of human experimentation is measured, at most, in litres, while the volumes of lakes or tide pools where life is thought to have originated must have been measured at least in cubic kilometers.

The first simplification which is made in almost all studies relevant to the origin of biological organization is to work with a simple mixture of pure organic compounds. In our own work we use D-nucleotides, other investigators use, for example, single L-α-amino acids or mixtures of L-α-amino acids. Prebiotic syntheses rarely, if ever, produce single substances or simple mixtures. Even the most plausible reactions, such as Miller's synthesis of amino acids in an electric discharge, produce numerous "non-natural" analogues and, of course, produce D- and L-enantiomers in equal amounts. Spontaneous reactions leading to sugars, nucleotides, etc. are far less specific. We know in many cases that the unwanted materials would interfere seriously with the reactions which we are able to demonstrate in the laboratory using pure reagents. Do these simple considerations invalidate all laboratory work on the origins of biological organization?

This is a hard question to answer. The environment on the primitive earth must have permitted extensive enrichment of particular classes of organic compounds on the basis of solubility, thermal stability, tendency to adsorb on inorganic surfaces, etc. Furthermore, reactions that could be relevant to the origins of biological organization vary widely in their sensitivity to inhibition by substrate analogues. However, even when these favourable considerations are taken into account, belief in the relevance of most laboratory experiments and most theoretical studies to the origins of life on the earth, involves, consciously or unconsciously, a good deal of faith.

At least one group of researchers, appalled by these difficulties, has proposed a radical solution—an inorganic origin of life based on self-replicating clays [7]. Any experimental demonstration of the main claim of the theory—that there are clay structures that act as primitive catalysts with the specificity of enzymes and that are also able to replicate accurately—would command the greatest respect. The absence of any scrap of experimental evidence twenty or more years after the first publication of the theory is one of the reasons why I find the new theory even less plausible than the conventional one that it is designed to replace.

2. Replication (theoretical)

Even the simplest forms of life are so complicated that their spontaneous appearance in a mixture of prebiotic organic compounds would constitute a miracle. We must conclude that contemporary cellular life was preceded by a series of systems of gradually increasing complexity. Natural Selection is the only mechanism that could have generated such a series of intermediates.

The theory of Natural Selection was, of course, conceived by Darwin and Wallace as an explanation of the origin of new species on the earth. However, the central dogma of natural selection—that those who reproduce most successfully eliminate all competitors—must apply to any family of objects that are capable of sufficiently but not perfectly accurate replication.

The objects that we will be interested in are macromolecules made up by arranging a set of related but distinguishable small molecules (monomers) in a determined order, for example, proteins and nucleic acids. A macromolecule replicates when it brings about the synthesis of a new macromolecule sharing the parental sequence. Variant progeny macromolecules that differ from the parental molecule at one or a few positions in the sequence are called mutants. Their appearance is inevitable, even if at very low frequency, and provides the variability on which natural selection depends.

The eventual evolution of complexity by natural selection in complicated chemical systems is not inevitable. If, for example, formaldehyde, a highly reactive and versatile molecule, is fed into a continuous flow reactor and the products are sampled from time to time, it is found that the product mixture soon reaches a steady state. The steady-state mixture is certainly complicated, but it shows none of the characteristic organization that interests us [8]. No mechanism for achieving very complex organization by natural selection seems to exist in this system.

In fact, the only method that we know to be able to generate increasing molecular complexity through natural selection is residue-by-residue replication. There may be other ways that do not involve residue-by-residue replication, for example through complex cycles of reactions in which each cycle generates molecules that play a part in other cycles. However, no detailed description of such a system of cycles has ever been offered, and I am sceptical that such systems are possible.

The arguments presented so far suggest that experimental study of molecular replication might provide models of an important step in the origins of life. But where should one start? One can draw on paper a very large number of potentially self-replicating macromolecules, and many of them have special features of interest to the chemist. What considerations guide the choice of a particular experimental system?

The student of the origins of life (on earth) should, I believe, require minimally that:

(1) The monomeric components of the system (or sufficiently close analogues) can be synthesized under prebiotic conditions;

(2) The proposed replication mechanism is compatible with and, if possible, supported by the established chemistry of the components.

It is also desirable, but perhaps not essential, that there be a reasonably close relation between the proposed primitive mechanism and contemporary nucleic-acid replication.

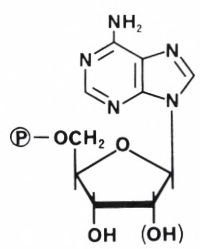
Even with these restrictions there remain a number of possibilities. The most conservative is the hypothesis that the self-replicating genetic molecule has always been a nucleic acid. Another possibility is that the first replicating polymer was related to a nucleic acid but had a different and simpler structure. It is possible, but in my opinion less likely, that life originated with a self-replicating protein or carbohydrate, or with something even more bizarre.

We have chosen to work with the standard nucleotides. In making this choice we have in part been guided by our belief that this is a very plausible first choice. However, there is another and far more important reason. The chemistry of nucleotides and polynucleotides is well-developed. A variety of starting materials are available commercially and, most importantly, enzymes can be used to synthesize new starting materials and analyze reaction products. These factors allow one to avoid the very extensive preliminary chemistry that is needed when one modifies the nucleic-acid structure in any way. Like the man who lost his watch on a dark night, we are forced to look where the light is; fortunately it seems a very good place to start.

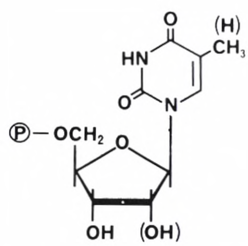
3. Template-directed synthesis

DNA and RNA replication as they occur in living systems are very complicated enzymatic processes. However, they always depend on the same basic chemistry. A single-stranded region of a preformed nucleic acid directs the synthesis of a complementary antiparallel strand. The four bases, A, U, G and C in the case of RNA direct the incorporation of the complementary bases U, A, C and G, respectively (fig. 1) via Watson–Crick base-pairing (fig. 2). For an excellent description of this fundamental aspect of molecular-biology the reader is referred to J.D. Watson's textbook [9].

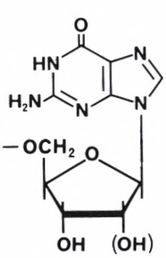
We have attempted to imitate this template-directed reaction without using enzymes. I will try to summarize very briefly the conceptual background of these experiments and the progress that we have made. However, to make the treatment accessible to the non-chemist reader, it will be necessary to neglect many important details.



Deoxyadenosine-5'-phosphate



Thymidine-5'-phosphate



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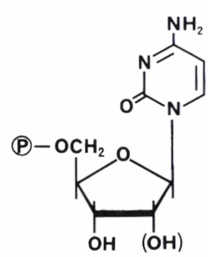

Deoxycytidine-5'-phosphate

Fig. 1. The structure of the monomeric components of the nucleic acids. The figure illustrates the deoxynucleotides, components of DNA. The corresponding ribo-nucleotides (components of RNA) have slightly different structures. The differences are indicated in parentheses.

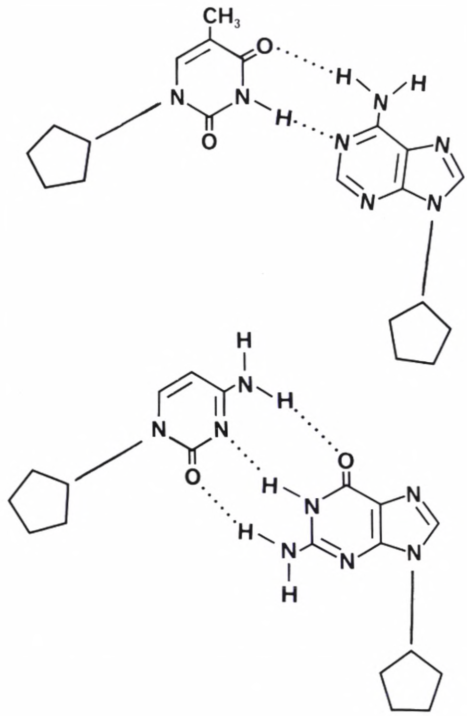

Fig. 2. The Watson–Crick base-pairs for DNA. Virtually identical pairing occurs between the bases in RNA double-helices and in RNA–DNA hybrids.

Todd Miles and Paul Ts'o and their co-workers discovered that a preformed strand of poly(U) or poly(C) was able to organize the complementary monomeric base or one of its derivatives into a helix with a structure closely related to that of DNA or RNA (fig. 3). This helix is stable only at low temperatures. The important point is that poly(U) organizes A derivatives into a helix while ignoring G derivatives, while poly(C) organizes G derivatives and ignores A derivatives. Poly(A) and poly(G), for well-understood reasons, do not organize U or C into helices.

The principle of template-directed synthesis is very simple. If the G derivative organized on poly(C), for example, is activated, it might zip-up rapidly if the arrangement in the helix brought the correct parts of the two adjacent monomers close together. Of course, activated derivatives also condense together in the absence of a template, but in dilute solution and without the orienting effect of a template this reaction is known to be inefficient and non-specific with respect to the nature of the bases involved.

It proved easy to demonstrate that poly(U) does bring about the polymerization of a variety of A derivatives, that poly(C) facilitates the reactions of G derivatives, and that neither template has any influence on non-complementary bases. The Watson–Crick pairing rules are obeyed [10]. However, it has proved much more

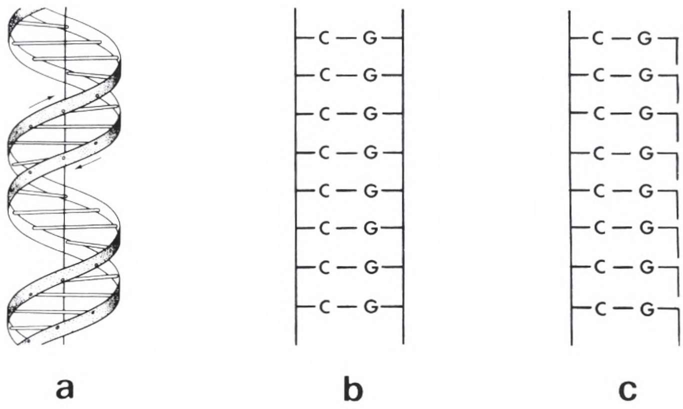

Fig. 3. The complementary structures formed between two polynucleotide strands or between one polynucleotide strand and complementary monomers. (a) Diagramatic illustration of the double-helices; (b) simplified view of a poly(G):poly(C) double helix; (c) corresponding diagram of a helix formed by poly(C) with a monomeric derivative of G.

difficult to achieve efficient copying of templates, particularly of those containing more than one base.

The activated nucleotide substrates in all enzymatic replications are tri-phosphates such as ATP. These substances are not suitable for laboratory investigation of non-enzymatic reactions because they react too slowly. Instead we use the imidazole derivative I, and the corresponding derivatives of the other bases (fig. 4). When I is used as a substrate, in the presence of poly(C), a very efficient polymerization occurs. The products are oligomers of G up to at least 30–40 units long that are identical to the naturally occurring substances. The reaction is highly selective—if one incubates a mixture of I and the corresponding derivative of another base with poly(C), the G derivative is incorporated 100–500 times more efficiently than the second base [11].

This is a good example of a template-directed reaction. In the absence of poly(C), the oligomerization of the G derivative is inefficient and yields a very complicated mixture of low molecular-weight products. When poly(C) is present long complementary oligomers are obtained in excellent yield. All bases except G are rejected by a poly(C) template.If one could extend this reaction to any arbitrary template, the

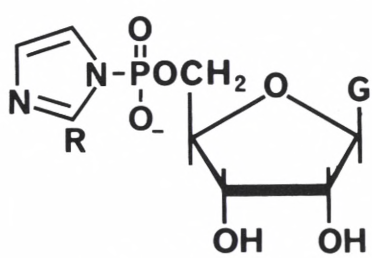
R = Me:2-MeImpG

Fig. 4. The imidazole derivate I; R = Me:2-MeImpG.

problem of non-enzymatic replication would be well on the way to solution. Unfortunately, this has not proved possible as yet. If one uses a template containing C and one or more other bases, one does indeed obtain oligomeric products that contain only the bases complementary to those in the template. However, the reaction is efficient only as long as C is the major component of the reaction mixture. For a variety of reasons, polymers containing less than 60% of C do not act as good templates in our system [12].

It has also proved possible to demonstrate "information-transfer" from template to product unambiguously in a few cases. The template CCGCC, for example, directs the synthesis of GGCGG without producing detectable amounts of isomers such as GGGCG. Unfortunately, the efficiency achieved so far never exceeds 20% [13]. Similarly the template d($C_3GC_3GC_3GC_3$) directs the synthesis of complementary oligomers up to $G_3CG_3CG_3CG_3$. The longer oligomers, however, are formed in yields well below 1% [14].

While these studies show unambiguously that the Watson–Crick pairing scheme is applicable to non-enzymatic synthesis of oligonucleotides, they also uncover a number of difficulties. First they show that the initiation of product synthesis is not restricted to the end of the template. A significant proportion of the products on a C_7GC_7 template, for example, are of the form G_6CG_n and G_5CG_n. A second difficulty is the stable self-structure formed by many templates which prevents further synthesis. The oligomer C_3GCGC_3, for example, has no template activity because it oligomerizes to form a stable mini-helix with four GC base pairs. A related difficulty is the stability of the template-product helix which must be dissociated before further reaction can occur. Finally, in our system, templates work efficiently only if they contain more than 60% of C. The complements, therefore, contain at most 40% of C and cannot act as templates. This is probably a technical difficulty that can be overcome by modifying the chemistry slightly.

4. *What do experiments on template-directed synthesis suggest about the origin of nucleic-acid replication?*

The overwhelming impression gained by studying a wide range of template-directed reactions is one of detailed, idiosyncratic, chemical complexity. No two templates behave in the same way; the products of template-directed synthesis even on a simple template are always complex mixtures containing oligomers of different lengths, isomers with different linkages etc. The principle of complementary base-pairing is obeyed, as in enzymatic synthesis, but beyond that all of the precision of the biological system is lost.

These difficulties might, of course, be due to an unfortunate choice of substrates, but I do not think this likely. It seems probable that the complexity of the product mixtures reflects the variety of chemical reactions that can occur between molecules as complicated as activated nucleotides. The enzymatic process of replication is neat and tidy only because it greatly accelerates one single sequence of chemical reactions while suppressing all other reactions. In the absence of enzymes it is unreasonable to expect a precise end-to-end copying of a template strand.

A more realistic picture is obtained by considering what would happen in a steady-state chemical reactor into which activated nucleotides are fed at a slow, steady rate, while a random mixture of substrates and products is extracted at an equivalent rate. At first a complex, "zero-order" mixture of short oligonucleotides with varying base-sequence and isomeric structure would begin to form, necessarily by non-template reactions. Among these oligomers would be some that are able to direct complementary synthesis. The composition of the mixture would then begin to drift away from that of the "zero-order", non-templated product mixture.

There seem to be two possible end points for this drift. Pessimistically, one might expect the system to settle down to produce a steady-state mixture not very different from the "zero-order" mixture; non-template-directed synthesis would remain dominant, and small amounts of inconsequential additional material would be produced by template-directed processes. The outcome expected by the optimist is very different—the reaction mixture might gradually change until template-directed synthesis became dominant. The final, steady-state product would consist of a complex mixture of oligomers very different from the "zero-order" mixture. No individual molecule would necessarily have the capacity to replicate accurately, but the whole family of molecules, through template-directed synthesis, could sustain itself with a composition very different from that of the "zero-order" mixture.

What reactions would be essential for the maintenance of a steady-state self-replicating system? Clearly one must incorporate new material into longer oligomers to compensate for the longer oligomers that are removed at random from the system. The primary source of new material, under prebiotic conditions is likely to be activated monomers, since most primary activation processes do not work efficiently on oligomers. Thus, template-directed incorporation of monomers is likely to be one fundamental reaction.

Since template-directed reactions do not always generate full length copies, it also seems necessary to have a second elongation process to ensure that the average length of the product molecules is maintained. The most plausible mechanism is the template-directed joining-together of short oligomers, a process called "ligation" in biochemistry. The reaction has been demonstrated as a non-enzymatic chemical reaction, for example by Naylor and Gilham [15] who showed that two T_6 molecules can be joined to make a T_{12} molecule on a poly(A) template. The intermediates in biochemical ligation, oligonucleotides capped with a pyrophosphate bonded A residue, are also likely prebiotic ligation intermediates, formed in secondary reactions from oligomers and activated monomers.

In principle, these two reactions are sufficient to maintain a "steady-state" system. However, an additional process is likely to have been important, namely the sequence-specific hydrolysis that generates free ends. The amount of new synthesis that can occur must depend in part on the presence of free ends, so hydrolysis that specifically produces free ends suitable for further elongation could have been very important for the evolution of an efficient replicating system.

Finally, one should mention the possibility that reaction cycles were important for prebiotic replication. Templates are likely to form stable complexes with their products which block further reaction. Repeated cycles in which the temperature is temporarily raised, and then lowered again, for example, could melt these complexes and allow synthesis to re-initiate. Cycles involving periodic changes in the pH, salt concentration, etc. could have a similar effect.

The final picture of replication that emerges does not have the elegant simplicity of modern replication. Instead, we have a picture of a complex mixture of macromolecules, sub-sequences of which are templates for the synthesis of intermediate-sized oligomers. These latter oligomers are mobile and can move to new sites where they can be extended by incorporation or joined by ligation. From time to time an oligomer breaks in such a way as to generate two rapidly growing fragments. A system of this type seems to me as close an approximation as one can expect to a modern replication mechanism in the absence of more specific catalysts.

5. RNA catalysts—Ribozymes

One of the most exciting discoveries of the last few years is that RNA molecules, without the help of proteins, can catalyze a number of chemical reactions. RNAase P is an enzyme that contains both a protein and an RNA moiety. In the presence of a sufficient concentration of Mg^{2+}, the RNA component alone will hydrolyze its substrate—a highly specific RNA sequence [16]. The cleavage occurs at a unique point in the substrate RNA.

The self-splicing of RNA is even more remarkable. The first system discovered involves the elimination of an intron from Tetrahymena ribosomal RNA. A central segment of the precursor RNA is eliminated, and the two end segments join together spontaneously. The mechanism is understood in some detail and depends on a series of trans-esterification reactions [17].

These examples of catalysis by RNA, together with laboratory experiments on template-directed synthesis, show that RNA catalysts (Ribozymes) and templates can bring about a number of interesting sequence-specific transformations of other RNA molecules and of the monomeric nucleotides. The key and as yet unanswered question is, "Can RNA molecules either alone or with the help of associated small molecules such as co-enzymes, catalyze reactions which do not depend on the direct base-pairing interaction of the catalytic RNA with a sequence of another RNA molecule?" If Ribozymes could catalyze either the replication of an arbitrary polynucleotide sequence or the reactions of intermediary metabolism, for example, biological organization could have evolved considerably before the invention of protein synthesis. This clearly should be a key issue in discussions of the origins of life.

6. The origin of the genetic code and of protein synthesis

Experiments designed to reveal the physico–chemical basis of the genetic code have proved inconclusive. Theoretical models seem to me to ascribe to short polypeptides properties that the real molecules are unlikely to possess. I suspect that the genetic code did not evolve as directly as most mathematical models propose.

The evolution of complex adaptations can only be explained by Natural Selection if there are many intermediate forms, each of which is already at a selective advantage. The selective mechanisms in the intermediate stages may or may not be related to the "purpose" of the final adaptation. In the case of the evolution of the genetic code it seems to me essential that the attachment of amino acids or short peptides to the 3′-termini of RNA molecules must have favored the replication of the RNA molecules prior to the evolution of protein synthesis. Polypeptides might, for example, have directed incoming activated nucleotides to the 3′-terminus of the template.

The most interesting recent work relevant to the origin of protein synthesis concerns the stage immediately after the appearance of the genetic code. Gilbert has argued persuasively [18] that contemporary RNA molecules contain in their sequences clues to the nature of the very earliest catalytic polypeptides. It appears likely that modern proteins have been formed by combining and recombining very primitive polypeptides usually made up of 30–40 amino acids. There are already clues that these original polypeptides are based on a few structural "themes". This is a rapidly developing field in which important discoveries can be expected. Hopefully they will serve to define the nature of the most primitive coded polypeptides.

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Discussion, session chairman N.K. Jerne

Weisskopf: In both examples you showed, you have used RNA, which is already a very complicated construction. I have two questions. First, how did Nature come to such a complicated thing, and are there other molecules thinkable that would do the same job?

Orgel: The experiments described would not necessarily show what happened historically on earth, but they would show a great deal about the various ways in which matter organizes itself. RNA seems an economical choice, and it is my guess that the original molecules were something similar.

Bjørnholm: You have shown so nicely how polynucleotides may replicate spontaneously. Do you think of the origin of life as a matter of nucleotides only, with the polypeptides (enzymes) at a later stage? After all, one may also view the cooperative functions of polynucleotides and polypeptides, forming feedback loops, to be the essence of life on the molecular level.

Orgel: I am inclined to think that the first thing to happen was the evolution of the self-replicating molecule, and that the manipulation of amino acids came as a subsequent development.

Fowler: I got the impression that in the molecular replication, when a mutation occurs it is always successful. Why can the opposite not occur?

Orgel: The impression that I wanted to give was that the successful mutations were those that were *retained*; neutral or disadvantageous mutations were eliminated.

Casimir: If you are able to inhibit the replication by means of added druggs, and mutations show up to overcome the effect of the inhibitive drug, how sure are you that the mutations are there anyway, unaffected by the presence of the drugs?

Orgel: This is a central point,. best described as "anti-Weissmanism": both the molecular "phenotype" and "genotype" are indeed affected.

Casimir: I may be influenced by my industrial past, but it seems to me the DNA can be regarded as a blueprint of a product to be manufactured. Customers may complain about the product and they may themselves make certain modifications, or service mechanics may do so. That is changing the phenotype. But if pressures are very large and the products do no longer sell, then the blueprint will be changed. So I am willing to believe that if a species is under extreme pressure this may stimulate the occurrence of mutations, although I know this is against the generally accepted dogma.

Broglia: Is there any way of getting an estimate, rough as it may be, of the timescales needed to produce a simple viable organism?

Orgel: I'm afraid the answer is "No".

Kristensen: Is it so that a condition for the evolution of new species is an environment that changes, but not too fast?

Orgel: That is probably correct if "environment" is taken to mean not only the abiotic, physical surroundings, but also, for instance, competition from other species.