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The Ribotype Theory on the Origin of Life

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The ribotype is defined as the ribonucleoprotein system of any cell. The theory substitutes the genotype–phenotype duality with the trinity genotype–ribotype–phenotype, and proposes that life on earth originated with the ancestors of today's ribotypes.

The first three chapters describe separate models on precellular evolution, the evolution of protocells and the nature of the cell respectively, and the unity of the theory comes from the fact that they form a consistent and interdependent whole.

The core of the theory is the ribotype hypothesis, of which two formulations are given. The restricted version is based on a link between ribotypes and ribosome biogenesis, and provides an explanation for the difference between 70S and 80S ribosomes. The general version describes a link between ribotypes and cell-types and explains why prokaryotes have 70S ribosomes, eukaryotes 80S ribosomes and endosymbionts a type of ribosomes similar to the bacterial ones.

If the creation hypothesis, panspermia and spontaneous generation are set aside, all alternative models of the origin of life belong to two schemes which are referred to as the genotype and the phenotype theories. It is shown that these theories rely on some discontinuity between past and present biological principles because of the need to break their inherent chicken-and-egg paradoxes, while the ribotype theory does not. Its hypotheses, free and arbitrary as they are or appear to be, have been built exclusively on properties and processes for which solid evidence exists, and the continuity between past and present biological laws is assumed as a corollary. Finally, it is shown that falsification tests are possible, and some of them are expected in the relatively near future.

1. The Origin of the Cells

1(A) THE PROBLEM OF THE ORIGINS

For more than a century, now, the problem of the origin of life has been formulated as the problem of describing the spontaneous generation of the first cells in the environment of the primitive earth. Since Darwin's time there have been shifts of emphasis from one aspect of the problem to another and a substantial increase in paleontological and geochemical evidence, but the essence has not changed.

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We divide the history of life, as Darwin's contemporaries did, into two great phases: a period of chemical evolution which virtually starts with the solidification of the earth's crust, and a period of cellular evolution which goes from the protocells on.

The study of the origin of life is concerned with what happened in between. How was it that spontaneously formed macromolecules managed to give origin to a co-ordinated supramolecular system which had the characteristics of a primitive cell.

Various models have been proposed on the subject, but when they are reduced to basics it turns out that they are all variations of two basic theories each of which represents, in a modern version, an answer to the old riddle of the chicken-and-egg paradox.

According to one theory, life started from naked genes—or primordial eggs—which later developed protective coats around them and gave origin to cells and organisms as their throwaway survival machines. This is the view which has been condensed in statements like "organisms are DNA's way of producing more DNA" or "the chicken is the egg's way of making another egg".

The other theory maintains that life began with coacervates of primitive proteins—or primordial chicken—which slowly evolved a replication strategy and eventually used DNA as a convenient storage of biological information. This theory reflects the common sense view that the egg is still the chicken's way of making another chicken, and not the other way round.

These are the two theoretical frameworks within which the problem of the origin of life has been formulated up until now. The choice of either scheme is obviously critical and in view of this it is important to examine two questions. Are we really sure that there is no other theoretical framework in addition to the above-mentioned ones? Is there any way of testing them?

The first question will be answered in the following pages by actually describing a third theoretical scheme. The answer to the second question, instead, will have to be left open.

It will be up to future developments to demonstrate which theory has implications which fit with the greatest number of available data and produces the most convincing description of the origin and the evolution of life.

1(B) THE THIRD APPROACH

As we have seen in the previous section, it is thought that the first cells originated either through a gene-path or through a protein-path according

to basic views which are referred to as the genotype theory and the phenotype theory.

It is easy to see why the problem of the origins has been formulated in such a way that only two alternative solutions appear to exist. Any biological organism is described as a duality of genotype and phenotype whose integration has been the result of millions of years of evolution and could not possibly have arisen from a single event of self-assembly. In this situation there is little choice but splitting the couple in two and choosing either the genotype or the phenotype as the side whose ancestors started life on the planet.

From this it follows that an alternative solution implies a revision of the genotype–phenotype category and I believe that this is not only possible but necessary.

The intermediary between genes and proteins is the system of ribonucleoproteins of the cell to which I give the collective name of "ribotype". I conceive an organism not as a duality but as a trinity "genotype–ribotype–phenotype" and propose that the ancestors of the present day ribotypes were at the origin of life on earth.

At first it is almost impossible not to feel a sense of artificiality about this proposal because the genotype–phenotype paradigm has such deep roots that it has become a sort of fundamental category of our description of nature. I do not propose to eradicate this category but to show that it is an ideal limit which can still be used in its established sense for many practical purposes, but not in all cases.

Another possible reaction is to compare the trinity of concepts with the sequence DNA → RNA → proteins which forms the central dogma but it will be noticed that the RNA of the dogma is messenger-RNA, not ribosomal-RNA.

The ribotype of a cell, instead, has its main representatives precisely in its ribosomes while messenger-RNA is, quantitatively, only a minor component of it.

The relationship between genotype, ribotype and phenotype cannot coincide therefore with the flow of biological information which is represented by the central dogma, and it will be shown later that it has indeed a more general character.

Finally, it may be objected that the ribotype cannot be conceived as an autonomous entity, because its instructions are inscribed in the genotype, while its structure and function are parts of the phenotype. Again, no quick answer can be given to this objection. Instead, I will develop gradually the theme that the ribotype has a reality of its own, in the very same sense that genotype and phenotype have theirs, and at this stage the reader is invited to keep an open mind.

1(C) THE PROGENOTE THEORY

The idea that ribonucleoproteins originated spontaneously and achieved relatively high levels of complexity during chemical evolution has been widely accepted for many years. It is generally believed, however, that what ensured in the end the survival of the ribonucleoproteins was their role as intermediaries between genes and proteins and it is the origin and development of these last components which are seen as the primary events of evolution. In respect to them the history of the ribonucleoproteins is regarded as the history of a necessary instrument, or little more. I believe that this view is biased, but the ideas which have been proposed on ribonucleoprotein evolution are nevertheless useful.

In this field one of the most important contributions has been made by Carl Woese and I have found it appropriate to summarize his main concepts before developing my own thesis.

At the centre of Woese's work there is a revolutionary model on protein synthesis which he proposed in 1970 and according to which the essence of translation lies in the interactions between allosteric loop parts of ribosomal and transfer-RNA (the ratchet model). At the time the ribosome was regarded essentially as a pack of enzymes and the ribosomal-RNA was considered a passive component which only serves as a scaffolding for the function-defining ribosomal proteins.

Woese's model virtually reversed the roles of the ribosome components. The proteins were declassified to the secondary task of facilitating translation and ensuring its accuracy, while the heart of the mechanism was shifted to the allosteric interactions between the ribonucleic acids. Later this model was used for evolutionary considerations.

If the core of the translation machinery is made up of relatively small fragments of ribonucleic acids, we can easily imagine that even smaller versions of these molecules could originate spontaneously in a primitive environment and became the first translation machines which appeared on earth. Crick *et al.* (1976), for example, have used Woese's ratchet model to devise an ingenious primitive coding scheme based on a pentanucleotide codon. Woese does not propose a model on the origin of the first cells and turns his attention instead directly to the evolution of the translation apparatus inside the protocells.

One particular characteristic of the apparatus—its extremely high accuracy—is not expected to have evolved in a precellular phase because it is the cell which defines its biological "raison d'être" and only the cell could have provided the framework and the habitat for its evolution.

Woese concludes, therefore, that the first cells that appeared on earth had to have a crude, noise ridden and error prone translation apparatus which was either a primitive ratchet mechanism based exclusively on ribonucleic acids or a slightly more complex version of it.

This implies that from the messengers of any one gene the translation apparatus would not provide identical copies of a specific protein but a class of "statistical proteins" which had only a group relationship with the original gene. Since a one-to-one relationship between genes and proteins is at the very basis of biological specificity, Woese concludes that in such primitive cells (which he calls progenotes) biological specificity was low, the proteins were presumably rather small, and the cells could only perform, erratically, the most elementary processes. The evolution of the progenotes, therefore, had to go hand-in-hand with the evolution of the translation apparatus towards higher levels of accuracy until a strict linkage between genotype and phenotype was achieved. Only at this point did biological specificity as we know it come into being.

The ribosomes of the first progenotes were low molecular weight structures and could not avoid errors simply because light molecular machines cannot avoid thermal buffeting. "There exists a direct correlation between the size of an automation—as measured roughly by number of components—and the accuracy of its function" (Burks, 1970). "To function accurately the ribosome must be nearly immune to thermal noise and so must be properly large" (Woese, 1980).

Ribosomes became, therefore, heavier in order to become more accurate and their evolution had to go all the way towards the high molecular weights which characterize their modern counterparts before what may be called eugenotic (as opposed to progenotic) cellular evolution could start.

There is some evidence which supports this conclusion. With experiments based on ribosomal-RNA sequence homologies Woese (1977) has shown that the ribosomal-RNA sequences tend to be highly conserved phylogenetically and that the differences between the ribosomal-RNAs of different cells are representative of discontinuities which occurred among the early ancestors of today's cells. This implies that at least the ribosomal-RNAs of the early cellular ancestors had molecular weights comparable to those of their modern descendants.

One may wonder what happened to the ribosomes during the rest of the history of life if they had already achieved a very high level of accuracy, and correspondingly of structural complexity, at the beginning of it.

The answer is that evolution has introduced variations mainly at the level of the dispensable components—the ribosomal proteins—and a sign

of this can surely be seen in the extreme variety of types, shapes and sizes which characterize the ribosomal proteins of different species.

Ribosomal-RNA, however, has much more stringent requirements and even minor mutations of its sequences tend to produce an all-or-none effect on translation. It is therefore the ribosomal-RNA which had to be conserved in its basic sequences if the translation apparatus was to maintain its original standards and we come back here to Woese's original concept: the idea that it is the ribonucleic acid which is at the heart of the translation mechanism.

1(D) THE MOLECULAR WEIGHT PROBLEM

The progenote theory is a model on the evolution of the first cells but provides a framework which any model on ribosome evolution has to take into account. The concept, for example, that the evolution from low to high molecular weight ribosomes took place between a relatively advanced phase of chemical evolution and an early phase of cellular evolution is a major contribution to our understanding of the history of life and I do not hesitate to subscribe to it. I cannot, however, agree with Woese's reconstruction of what happened during that period.

A critical point is the idea that the increase in molecular weight of the ribosomes, and in particular the acquisition of most of their proteins, was favoured as a means to avoid thermal noise and increase the translation efficiency of the machine. This concept must contain *some* truth but cannot be the whole truth.

The molecular weight of the 70S ribosomes is over two millions while most 80S ribosomes exceed four millions and yet do not have, because of this, a greater translation accuracy.

An increase in molecular weight of almost two million daltons not only does not bring about any increased protection against thermal noise but definitely results in a very heavy metabolic burden since the production of ribosomes takes a substantial share of the energy and material resources of the cell. Natural selection should have therefore strongly favoured the mutations which adjust the ribosome weight to the minimum level which is compatible with its highest degree of accuracy. We know that such a level is not much greater than a two million molecular weight size and yet most cells have ribosomes which are almost double that size, despite the fact that the mutation frequency of the ribosomal genes could have allowed the weight readjustment many times over.

I conclude that the increase of the molecular weight of most ribosomes cannot be explained *exclusively* by the need to avoid thermal noise. This is one of the reasons why I had to abandon Woese's theory and later on

I will show that my solution to the molecular weight problem does require a substantially different approach.

At this stage the difference between the two theories can be summarised as follows. The progenote theory describes the evolution of the translation machinery as the evolution of the link between genotype and phenotype and is bound therefore to assume that the main course of such evolution took place within the cell. The ribotype theory, instead, proposes that the evolution of the ribosomes was instrumental in bringing about the very origin of the cell and the crucial events had therefore to take place at the precellular level.

In the remaining sections of this chapter, and in those of Chapter 2, the ribotype reconstruction of the origin of life will be done by describing a series of phases which are, individually, highly arbitrary. When, however, the events of precellular and cellular evolution are arranged in a complete sequence it will be possible to see that they form a unitary pattern and in Chapter 3 the ribotype theory will be discussed as a whole.

1(E) RIBOSOIDS

For convenience, the term ribosoids will be used to indicate either ribonucleic acids or ribonucleoproteins.

One has only to think of the ease with which polynucleotides and polypeptides can interact, and to the endless number of combinations which can arise from them, to realize that a wide range of simple ribosoids could well have appeared on earth at a relatively early phase of chemical evolution.

Furthermore, I will borrow Woese's concept that molecular machines for protein synthesis, based on the ratchet principle or on equivalent mechanisms, could and did originate spontaneously in the primeval solutions. Not only did these structures have simple components but their overall organization is based on straightforward self-assembly properties and there is no need to regard them as the result of unlikely accidents.

There is therefore a widespread acceptance of the idea that some form of protein synthesis was performed in the primitive open systems long before the origin of the first cells. Once this principle is established, however, attention is usually shifted immediately to the protein syntheses which later took place within the protocells.

The rationale for this jump is that the transition had to take place anyway and, since we do not have enough information to reconstruct it, it is better to go directly to the next phase without indulging in unnecessary intermediate speculations.

As has happened only too often in science, the sensible desire to avoid making explicit hypotheses about elusive phenomena is usually implemented by making an implicit one which is far more dangerous because it unconsciously conditions our reasoning.

In this case the implicit hypothesis is that the protocells somehow originated independently and used the ribosoids of their surrounding environment as instruments for intracellular protein synthesis only. This is already a definite conclusion about the origin of life and it should not be taken for granted simply because it appears to be a sensible one.

I propose therefore to discuss in some detail what might have happened between the formation of the first ribosoids and the origin of the first cells.

The ribosoids which had the ability to join aminoacids together by using a ratchet-like mechanism or equivalent ones were necessarily simple and low molecular weight machines, at first. I will not even call them translation machines because they could not possibly translate a polynucleotide with any accuracy and because the genetic code had not yet been developed. They were simply polymerizing ribosoids, endowed with the ability to stick aminoacids together and produce totally random proteins. A translation mechanism would not even have much sense at this stage: the messengers of the open primitive solutions were bound to be random sequences of nucleotides and their translation would have resulted in any case in the production of random sequences of aminoacids.

One may notice that we do not need ribosoids to produce random polypeptides because the spontaneous processes of synthesis which had generated the ribosoids in the first place, among various other compounds, were still operational. This is true, but one cannot fail to appreciate that polymerizing ribosoids could speed up the rate at which random polypeptides were formed enormously, and this is very important.

The conditions were created for the transformation of the primeval solutions from diluted into enriched systems where a wide range of proteins were present.

In this situation we do not need to rely on improbable accidents in order to assume that proteins of a particular class were formed; given a large enough variety these were bound to originate with percentages and frequencies which are determined by statistical factors only.

Some of these proteins, for example, were bound to be of the kind which favours the condensation of nucleotides and provided therefore the means for accelerating the production rate of all sorts of nucleic acids. Others were of the type which interact with ribonucleic acids to produce ribosoids and among the newly formed ribosoids some had polymerizing properties similar to those which had started the previous cycle of synthesis.

It is important to realise that the polymerizing ribosoids need not have identical structures in order to perform the same function. Ribosomes of different species can have very different components and yet they all translate a messenger with the same accuracy, as if they were identical. A similar polymorphism or polyvalency can be attributed to the primitive versions of the ribosomes and we can well conclude that the ability to polymerize aminoacids was an inherent property of an heterogeneous class of ribosoids. A ratchet mechanism, for example, can be conceived with an endless number of individual structural variations which all provide the same basic function.

We come therefore to a first major conclusion on the precellular history of life. A form of macromolecular replication based on the structural polymorphism of the ribosoids was possible long before the appearance of replicating cells. This replication strategy will be referred to as "quasi-replication" because the descendants are not exact replicas of the progenitors.

The polymerizing ribosoids, or protoribosomes, were capable of quasi-replication because they could produce at random a wide variety of products some of which could self-assemble themselves in new protoribosomes which had the same function of their predecessors even if their detailed structure and composition were different.

A quasi-replicating system has three main characteristics. The first is that only a fraction of its products are reinvested in the reproduction of the system. The second is that the original system is reproduced by the self-assembly of its individual components. The third is that the components which reproduce the system need not be carbon copies of the components of the original system.

I believe that what we now call biological replication, or carbon copy replication, is a mechanism which did not arise all of a sudden but was the long term result of the evolution of quasi-replicating systems.

The precellular history of life becomes in this way the history of quasi-replicating systems which went through various states of increased complexity before they could perform the transition into proper replicating organisms. And the ribonucleoproteins were at the heart of the quasi-replication strategy.

1(F) RIBOSOID EVOLUTION

According to Woese the evolution from low to high molecular weight ribosomes took place within the protocells because it was favoured by natural selection as a means of providing an increasingly accurate linkage

between genotype and phenotype. I propose to approach the same problem with a different spirit and simply to ask if high molecular weight ribosomes could have evolved at a precellular level or not.

A conventional answer to this problem would presumably be seen like this: the chance formation of any structure has a degree of probability which is inversely proportional to its degree of complexity, and since high molecular weight ribosomes are among the most complex compounds of Nature their spontaneous generation has correspondingly a very low degree of probability. This implies that once a heavy ribosome is formed, a considerable amount of time would have to elapse, on average, before a second one could appear. But highly complex structure like ribosomes are inherently unstable and it is likely therefore that any one ribosome would have degenerated long before another one had the chance to be formed. The conclusion is that heavy ribosomes could well have appeared at a precellular phase but one cannot see what sort of an impact they could have had on evolution. This sort of reasoning has two faults: a minor and a major one.

The minor bias is that the probability of the chance formation of ribosome is not related to its degree of complexity only. Such a function must be multiplied by the number of different ways which can give origin to a ribosome and this is indeed a very high number since ribosomes of different species can differ in a wide range of parameters and components. Furthermore, ribosomes can self-assemble themselves from their individual components and this also affects the degree of probability which has to be associated with their spontaneous formation.

The major bias lies in a purely kinematical representation of the phenomenon, as if ribosoids could only have given origin to individual particles which were uniformly scattered in the primeval solutions as the free diffusing molecules of a gas. When the ribosoid tendency to form aggregates is taken into account an entirely different situation arises as will be shown in the following sections.

1(G) NUCLEOSOIDS

Ribonucleoproteins and ribonucleic acids can form a variety of supramolecular aggregates and it is only natural to assume that clusters of ribosoids could and did originate in the primeval solutions, particularly when these became enriched by ribosome-driven synthesis of proteins and enzyme-driven synthesis of polynucleotides.

The limits to the dimensions of such aggregates are anybody's guess but the best example that we have nowadays is represented by the nucleoli, and because of this I will call them "nucleosoids".

Nucleosoids are, therefore, coacervates of ribosoids, or of ribosoids and other compounds, and represented a highly heterogeneous family whose members had a variety of sizes, shapes, dimensions and properties. Since their formation was essentially a random process I do not hesitate to recognize that most of them were inert or nonsense structures and represented dead-ends from the point of view of evolution. The same statistical argument, however, implies that if there were clusters of ribosoids which had useful biological properties they were bound to appear with a defined frequency.

The comparison with modern nucleoli should not be taken literally but it is nevertheless useful for extracting general properties which can be attributed to a wider class of aggregates. What the nucleoli do show us is that a cluster of ribosoids is not an inert scaffolding. For one thing its ribonucleoproteins can perform physical movements from one point in space to another and undergo a variety of conformational changes despite their arrangement in supramolecular aggregates. Secondly, clusters of ribosoids provide microenvironments which trap macromolecules and localise or compartmentalize their interactions. Thirdly, they form three-dimensional backbones which can reach dimensions of the order of the micron and which can be used as the support structure for a variety of processes which need supramolecular substrates to take place.

Three-dimensionality as well as functional and structural plasticity can be regarded as inherent properties of the nucleosoids and those combinations which happened to express them best were bound to be favoured by natural selection.

It may be pointed out that nothing is of any value in evolution if it has no lasting effect and however interesting the nucleosoids were, one cannot attribute to them replication properties. The answer is, once again, quasi-replication.

The nucleosoids could have a microcosmic system of synthesizing ribosomes and ribonucleic acids which could serve as templates. The very fact that they could trap only a limited amount of transfer-RNAs was probably sufficient to select a genetic code and at this stage it did not matter if different nucleosoids were using different codes.

The important point is that nucleosoids could synthesize a variety of compounds and their supramolecular organization could trap in a confined space the synthesizing ribosoids so that the cycles of synthesis could go on for a long period of time. Most of the nucleosoids were presumably not making the right kinds of syntheses from an evolutionary point of view but on purely statistical grounds one can assume that a fraction of them were

preferentially synthesizing other ribosoids and these were bound to have a future.

A nucleosoid in which the synthesis of its own components goes on can in fact grow on itself and reach whatever dimensions are physically attainable. Eventually, however, it would become unstable, break apart in smaller pieces and in some of these the ribosoids which were responsible for the previous syntheses would simply go on repeating the original process. As in the case of the ribosoids, the quasi-replication of the nucleosoids is based on the fact that the descendants do not have to be carbon copies of the progenitors.

We know that nucleoli of different species differ in a wide range of parameters, properties and components and yet they perform the same function as if they were identical. It is legitimate, therefore, to assume that the primitive nucleosoids were also polymorphic and all that was required of them to produce descendants was the ability to synthesize components of their own class which could self-assemble into an organization similar to that of their progenitors. More precisely, among all the synthesizing nucleosoids those which produced other ribosoids could grow and give origin to descendants which repeated a similar cycle. The others could not, and were bound to become a minority even if at the beginning they represented the majority of the synthesizing nucleosoids.

1(H) HETEROSOIDS

The evolution of the nucleosoids was bound to change, in the long run, the overall macromolecular composition of the primeval solutions. The phase in which ribosoids quasi-replicated other ribosoids and protein synthesis was characterized by complete randomness was followed by a phase in which nucleosoids quasi-replicated other nucleosoids and protein synthesis was preferentially oriented toward the production of the nucleosoid components.

The ideal limit of this evolutionary trend may appear to be a system where nucleosoids produce exclusively their own ribosoids but this is not a realistic outcome. The nucleosoids inevitably produced a variety of heterogeneous compounds and if some of these were useful for quasi-replication purposes they were bound to be favoured by natural selection even if they were not typical ribosoids. One of these compounds, for example, is represented by membrane structures. Even if there was no way of ensuring the reproduction of a membrane system from one generation of nucleosoids to the next, if the association of a membrane had selective advantages the percentage of membrane protected nucleosoids was bound, on average, to increase with time.

The most important sort of "contamination", however, was another substance: DNA. Chains of DNA were only too likely to exist in the primeval solutions and could also have been produced inside the nucleosoids because of the intrinsic heterogeneity of the nucleosoid reactions. Up to now DNA has not been taken into account because it is a rigid one-purpose molecule while a quasi-replication strategy has necessarily to rely on polymorphic properties like those which are typical of the ribosoids. DNA, however, is an ideal parasite, and when the quasi-replicating systems of the nucleosoids were developed its exploitation of them was virtually inevitable.

In order to quasi-replicate themselves the nucleosoids have to carry instructions for the production of their ribosoids and to this purpose RNA is sufficient. DNA, however, can substitute RNA as a depository of instructions and at this job it performs better than RNA. Its high stability becomes now its greatest asset and the nucleosoids which contained a core of DNA had a selective advantage over the others.

There were, therefore, two major developments during the evolution of the nucleosoids. Inside them DNA started substituting RNA, and on the outside membranes started surrounding them with protective coats. The relationship between ribosoids and non-ribosoidal components became increasingly strict, and I summarise this process by saying that the nucleosoids became heterogeneous nucleosoids or heterosoids. The first heterosoids, however, were not yet the first cells.

Their quasi-replication mechanisms were still dividing them into unequal parts and a new phase of evolution had to take place before the advantages of producing equal descendants could operate an effective selection and proper replicating systems could finally emerge. When this phase was also completed, precellular evolution came to an end and the first cells appeared on earth.

2. The Evolution of the Cells

2(A) FOUR SCHEMES FOR CELLULAR EVOLUTION

One of the great generalizations of biology is that life exists on earth in two different cellular forms—prokaryotic and eukaryotic—and with this concept there are, in principle, four approaches to the problem of the cell origin and evolution.

The first is the possibility that prokaryotes and eukaryotes arose independently from precellular forms, possibly in environments which were at first physically separated from each other. This hypothesis is disregarded at

present because all cells share the same genetic code and a variety of biosynthetic mechanisms which strongly indicate a monoancestral origin.

The second approach is that the first cells were primitive eukaryotes from which the simpler prokaryotes derived through the escape of organelles which had sufficiently evolved to acquire a cellular status of their own. Even this view (the eukaryote theory) is regarded as a logical possibility only and generally set aside as a far-fetched hypothesis.

We are left, therefore, with the alternative that the common ancestor of today's cells was either a primitive prokaryote (the prokaryote theory) or a cell type which had neither prokaryotic nor eukaryotic characteristics (the akaryote theory).

2(B) THE PROKARYOTE THEORY

Paleontological and geochemical data indicate that the first cells appeared between three and four billion years ago while organism evolution started after the earth's atmosphere became aerobic, between one and two billion years ago. The cellular phase of evolution lasted, therefore, approximately two billion years, a period during which the cells developed predominantly in anaerobic conditions and photosynthetic microorganisms slowly built up a reservoir of oxygen around the planet.

There is little doubt that these microorganisms were prokaryotic cells, not only because their descendants still use a variety of photosynthetic mechanisms which are considered primitive, but also because the only photosynthetic apparatus of the eukaryotes derived in all probability from prokaryotic ancestors.

It is equally beyond dispute that a large scale operation like the transformation of the earth's atmosphere required the effective colonization of the globe by the prokaryotes and their diversification in a wide variety of types. The evidence for this conclusion comes from the fact that rock layers left behind by primitive bacteria (stromatolites) and deposits of fossilized bacteria of precambrian origin have been found in all five continents and reveal, as in the classic case of the Gunflint deposits, a wide variety of bacterial types, shapes and forms.

The fossil records do not imply that the prokaryotes were the only existing cells during the anaerobic phase of life, but such a conclusion appeared to many biologists a most natural one, and the first period of cellular evolution was described as a pure "Age of Prokaryotes". All models which share this view will be considered here as versions of the same "Prokaryote theory", however different their details.

The theory implies not only that eukaryotes evolved from prokaryotic predecessors but also that the very first cells which appeared on earth were

the direct progenitors of the prokaryotic tree and were therefore phylogenetically primitive prokaryotes themselves.

Within this framework attention has been focused on one main problem: how did the eukaryotes originate from prokaryotes? The most popular answer is represented by a suggestion which was first proposed at the turn of the century and which has been revived by Margulis in the late sixties: the symbiosis model. According to this hypothesis, chloroplasts and mitochondria were once independent cells which became engulfed by a third type of prokaryote and eventually lost their cellular status to acquire that of subcellular organelles.

This explains the acquisition of only two typical eukaryotic structures but the proponents of the prokaryote theory believe that the same mechanism also led to the acquisition of others.

Margulis, for example, has proposed that the symbiosis of spirochete-like prokaryotes brought about the acquisition of microtubules which are at the basis of cell locomotion and at the very heart of mitosis.

One of the most important consequences of the symbiosis model in particular and of the prokaryote theory in general is that the origin of the eukaryotes had to take place at a late, probably at a very late, phase of cellular evolution.

This is because such an event had to be preceeded first by the evolution of the prokaryotic cell organization and then by its diversification in a wide variety of different types which evolved separately and eventually became the progenitors of the various eukaryotic organelles. The conclusion is also reinforced by the widely held view that the precursors of the mitochondria flourished very late, when the world was already becoming aerobic.

The age of the prokaryotes lasted therefore, if the theory is correct, for a substantial part of the two billion years or so of cellular evolution, which makes the prokaryotes the sole inhabitants of the earth for almost half of its history.

This implies that the more complex cellular organisation of the eukaryotes was developed in a fraction of the time which the development of its predecessors took, but this is not necessarily an obstacle. The acquisition of endosymbionts, for example, is potentially a fast process and a recent development has even provided experimental support for this view. Kwang Jeon has observed in the laboratory that a colony of amebae, which had become infected by bacteria, produced within five years—less than 1000 generations—descendants which contained the bacteria as permanent endosymbionts.

It should be noticed that the host in this case was an eukaryote but the speed of the transformation, which included genetic readjustments, does,

nevertheless, indicate that endosymbiosis can account naturally for a rapid tempo of eukaryotic evolution.

Another version of the prokaryote theory is the model proposed by Nass (1969) which uses cell fusion instead of symbiosis as the key mechanism for pooling together different types of prokaryotic cells. This model has never been as popular as the symbiosis model but provides an equally fast process for the origin of the eukaryotes.

2(C) THE AKARYOTE THEORY

Many biologists have rejected not only the idea of a rapid evolution of the eukaryotic cell but also the very concept that eukaryotes originated by direct filiation from prokaryotes (Stanier, 1970; Cavalier-Smith, 1975; Woese & Fox, 1977*a*; Darnell, 1978).

Stanier, for example, has emphasized that the concept of endosymbiosis cannot be based on typical prokaryotic properties: "The impenetrability of the prokaryotic cytoplasmic membrane by any object of supramolecular dimensions effectively precludes the acquisition of endosymbionts" and "a stable endosymbiosis in which the host is a prokaryote has never been described".

These and similar arguments are indirect objections which could perhaps be circumnavigated with convenient hypotheses but there are also more convincing indications. Woese and his group, for example, have shown that prokaryotes and eukaryotes have a comparable phylogenetic antiquity and this brings the attack to the very heart of the prokaryote theory. With experiments based upon ribosomal-RNA sequence homologies, a sort of molecular genealogical analysis, Woese has demonstrated first that the prokaryotes comprise two different phylogenetic groups or kingdoms (eubacteria and archaebacteria) and, second, that none of these groups is any closer to one another than either is to the eukaryotic one. This means not only that we cannot put archaebacteria and eubacteria on the same phylogenetic line but also that neither of them can be considered the phylogenetic predecessor of the first eukaryotes.

The result is that we have three independent lines of descent which evolved in parallel and, if we accept the monoancestral postulate, all three had to derive separately from a common ancestor.

The characteristics of the ancestral cells are hypothetical but all authors agree that they did not have proper nuclei and therefore had the structure of simple prokaryotes. In this sense we could still say that eukaryotes derived from prokaryotes but such a statement would inevitably generate confusion because the term prokaryotic is used not only in a structural but also in a phylogenetic sense.

The identification of the first cells with primitive prokaryotes has, therefore, the explicit phylogenetic meaning that the prokaryote theory attributes to it, and if we want to say that the common ancestor had potentialities which were no more prokaryotic than they were eukaryotic we obviously need a new term. I will use the word "akaryote" (without a nucleus) for this purpose with the understanding that such a term is used exclusively in a structural sense to identify any cell which lacks a proper nucleus irrespective of its phylogenetic characteristics.

All models which state that eukaryotes did not originate directly from prokaryotes but that both cell types evolved independently from ancestral anucleated cells will be considered here as different versions of the same "Akaryote Theory".

A common feature of these models is that the development of proper nuclei is regarded as a late evolutionary event and it is, therefore, generally agreed that the earth was inhabited by anucleated cells for the greatest part of cellular evolution.

The concept of the age of the prokaryotes therefore remains, except that it should more properly be referred to as the age of the akaryotes, the different name implying that it was a period characterized by a plurality of distinct philogenetic groups of cells and not by one group only.

Another concept of the prokaryote theory which remains is that of endosymbiosis. As a matter of fact the akaryote theory provides a better explanation for it because for all that we know endosymbiosis is compatible only with the characteristics of the eukaryotic cytoplasmic membrane.

There is, however, one major difficulty which arises only in the context of the akaryote theory and which is potentially capable of undermining its credibility. Without chloroplasts and mitochondria, the precursors of the eukaryotes (the cells that Woese calls urkaryotes) were dependent upon glycolysis for their energy supply and this has two major implications.

First, it is unlikely that such cells could have progressed far in their path towards cellular complexity with only such an inefficient mechanism at their disposal. Second, the dependence upon glycolysis alone was bound to put them at a disadvantage against the greater range and versatility of the metabolic mechanisms of the prokaryotes. For the whole period which preceded the acquisition of the organelles the emerging eukaryotes lived under the constant threat of being selected against on energy competition grounds, and it is unreasonable to assume that such a precarious state could have lasted for a substantial interval of the geological time as the akaryote theory implies.

The most convincing and elegant solution of this problem was offered by Stanier (1970). He proposed that the critical event in the evolution of

the primitive eukaryotes was the acquisition of endocytosis (phagocytosis and pinocytosis) which allowed them to obtain nutrients for glycosis from other cells. By becoming predators, the emerging eukaryotes solved once and for all the problem of their food and energy supply and natural selection started working on them on new grounds, favouring the mutations which improved their predatory attributes.

The beauty of this model is that it explains naturally a general increase in cell size and the development of typical eukaryotic structures like the microtubular system and the Golgi apparatus as a means for promoting the active locomotion of the cell for hunting (cilia, pseudopodia) and the ability to capture, devour and digest the prey.

At the same time the microtubular system, once developed, provided a precondition for the eventual evolution of the machinery of mitosis. In addition to all this, the model explains endosymbiosis as a natural complement of the predation machinery. The ability to phagocyte other cells could have been dissociated from the necessity (or from the ability) to digest them and the engulfed cells would have survived within the host.

Stanier's model, in conclusion, not only explains how the primitive eukaryotes solved their energy problem, but allows one to see a unitary trend at work behind the development of typical and yet heterogeneous eukaryotic structures. It was in fact Stanier's model in 1970 which gave the akaryote theory its scientific credibility long before the decisive discovery with which Woese, in 1977, dismantled the basic assumptions of the once favoured prokaryote theory.

2(D) A NATURAL DICHOTOMY

The reconstruction of the history of prokaryotes and eukaryotes is complicated by the fact that there is not a general agreement on their nature and on the nature of their differences. On the contrary, the existence of a dichotomy among the two kinds of cells has been the object of a major dispute among biologists. Some have proposed that there is really a sort of continuum of cell types between the simplest and the most complex forms, but this unitary view has become increasingly difficult to defend. The structural and developmental gulf between prokaryotes and eukaryotes has become wider with the years, not smaller, and Stanier, Doudoroff & Adelberg (1963) have described it as "the greatest evolutionary discontinuity in the biological world".

A different solution to the dispute has been proposed by Woese & Fox (1977*b*) with the demonstration that the basic phylogenetic groups were three and the suggestion that a tripartite division of the cells effectively

rules out the existence of a natural dichotomy among them. It should be noticed, however, that two of these groups—archaebacteria and eubacteria—are made of prokaryotic cells and, in a structural sense, at least, the dichotomy remains. The phylogenetic argument could well reflect only a subdivision of cell types which is not incompatible with a structural and functional interpretation of the dichotomy. (An analogous case is sex. The discovery that there are genetic combinations which differ from those which are typical of male and female does not allow one to conclude that sex is no longer based on a natural dichotomy.)

One of the strongest arguments which has been put forward by the advocates of the dichotomy is that the relationship between transcription and translation follows two different general patterns in prokaryotes and eukaryotes. With a nuclear membrane, transcription is separated in space and time from translation while without it transcription is physically linked to translation in the sense that messengers can be translated at one end while their transcription still goes on at the other.

The linkage between transcription and translation has, for example, implications for the mechanisms which regulate protein synthesis. A strict linkage is compatible with short-lived messengers while the separation of transcription from translation is not, and long-lived messengers have to be employed.

Furthermore, a strict linkage requires an "open" arrangement of the prokaryotic genome, in the sense that all its genes must be almost directly accessible to the ribosomes, and this has consequences which can hardly be overestimated since it sets a limit to the total number of genes that the cell can carry. The size of a prokaryote would have to be simply enormous if it had to carry the number of genes of a typical eukaryote in a loose, ribosome accessible configuration.

When, however, transcription is separated from translation, the genes are no longer required to be directly accessible to the ribosomes which translate their messengers and can, therefore, be stored in tighter configurations which potentially allow an overall increase of the cell genome. Such a potential can obviously be exploited only if the replication problem is solved and this has required nothing less than the development of a new mechanism for cell division.

Once mitosis was developed, the eukaryotes could exploit their potential to carry high size genomes and the way was open for the emergence of multicellular organisms capable of true differentiation. Even the prokaryotes can form colonies of cells but these are in all cases microscopic forms of life which show little or no differentiation. Only the eukaryotes broke the microscopic barrier, gave origin to aggregates of billions of cells and

reached the dimensions of the large plants and animals which populate the earth.

It appears, therefore, that we can reach the following generalization: prokaryotes are cells where transcription is physically linked to translation; eukaryotes are cells where transcription is physically separated from translation. This is an interesting statement, but unfortunately the linkage between transcription and translation can only be an expression of the dichotomy between prokaryotes and eukaryotes, and not the cause of it.

Nature is not anticipatory. Natural selection could not have favoured the developments which resulted, for example, in the separation between transcription and translation because of the future advantages that such separation would bring.

We are back to the original problem: what caused the divergence between prokaryotes and eukaryotes?

2(E) THE RIBOTYPE HYPOTHESIS (RESTRICTED VERSION)

In Chapter 1 I briefly mentioned that the high molecular weight of the ribosomes cannot be explained exclusively by Woese's suggestion that it is a means of avoiding thermal noise and ensuring translation accuracy. This argument alone would not explain the very considerable molecular weight difference between 70S and 80S ribosomes.

Here I will reformulate the problem in the framework of the dichotomy between prokaryotes and eukaryotes: why is there no eukaryotic cell with 70S ribosomes? By utilizing such ribosomes an eukaryote would save almost 50% of the aminoacids and nucleotides which are invested in its ribonucleoprotein production and perhaps more if we include in the count the RNA which is discharged during 80S ribosome biogenesis.

The objection that eukaryotes simply happened to evolve that way is not valid because the mutation frequency of their ribosomal genes would have allowed an overall readjustment of the ribosome size many times over during evolution, and surely natural selection would have favoured mutations which reduce the metabolic burden of the cell without affecting its translation accuracy. My answer is that nature did try the experiment, and very likely in all possible ways, but without success: no cell could substitute 80S ribosomes with 70S ribosomes and still remain an eukaryote.

I propose that the transport of ribosomes from nucleus to cytoplasm depends upon the ribosome biogenesis processes of the 80S particles and is incompatible with a 70S type of biogenesis. This is the ribotype hypothesis (restricted version).

A eukaryotic cell with 70S ribosomes could not survive as a eukaryote because it would be unable to shift its ribosomes to the cytoplasm. A

prokaryotic cell, on the other hand, could live with 80S ribosomes but the metabolic burden of their production would make the cell unable to survive the competition of the prokaryotes with 70S ribosomes.

The ribotype hypothesis therefore explains in a straightforward way why all eukaryotes have 80S ribosomes and all prokaryotes have 70S ribosomes. Furthermore, the hypothesis indicates a natural solution to the molecular weight problem. The discrepancy between eukaryotic and prokaryotic ribosomes is inexplicable if we associate the molecular weight with translation requirements only, but not any longer if we say that ribosome biogenesis also contributes to the structure of the ribonucleoproteins. It must be emphasized that this explanation does not imply that 80S ribosomes have some components which are related to translation and others which are related to biogenesis. It simply states that a mature ribosome is the final product of a unique type of biogenesis and we cannot have, for example, 70S ribosomes with an 80S type of biogenesis. The eukaryotes, in other words, had to "choose" first an 80S type of biogenesis in order to be able to shift ribosomes from nucleus to cytoplasm and after that they were bound to have 80S ribosomes simply because these are the only possible final product of such biogenesis. (Later I will reformulate this statement in the light of a further generalization but for the time being I will use it as it is simply because it has a useful intrinsic logic.)

An experimental test of the ribotype hypothesis is not yet within our reach because it implies a detailed elucidation of the structure and function of the ribosome components, a comparative analysis of the eukaryotic and prokaryotic ribosome biogenesis and a precise account of what produces the transport of the eukaryotic ribonucleoproteins from nucleus to cytoplasm.

I believe, however, that sometime in the future the technical difficulties which are behind these problems will be solved and experimental tests will be possible. For the time being, I will assume that the ribotype hypothesis is correct on the purely theoretical ground that it has the ability to solve the molecular weight problem and to explain naturally the fact that each cell type has a typical class of ribonucleoproteins and a typical kind of ribosome biogenesis. Briefly, each cell has a characteristic ribotype.

With the ribotype hypothesis we are now in a position to approach the problem of cellular evolution from a new point of view.

2(F) THE ORIGIN OF THE PROTOCELLS

In this section I will continue the ribotype reconstruction of the early history of life from the point where we left it at the end of Chapter 1. It

will be recalled that precellular forms of life evolved from aggregates of ribonucleoproteins which were capable of producing descendants by quasi-replication and the first cells developed from these supramolecular centres of biological activity.

The picture of the protocells which emerges from this reconstruction is that of little bags which probably had the dimensions of small nuclei and which contained cores of ribonucleoproteins somewhat similar to nucleoli. At this point I raise an apparently unanswerable question: what kinds of ribosomes were present in the protocells? In general such questions are avoided by biologists but I believe that any model on the nature of the cells contains implicit assumptions about its ribosomes and it is preferable therefore to bring the problem out into the open.

Carl Woese was the first biologist who explicitly stated that the evolution of the translation apparatus is central for the evolution of the cell and after him the problem can no longer be ignored. Woese has made three major contributions which can be summarized as follows.

(1) The ribosomal nucleic acids changed little during evolution and their characteristics reveal the phylogenetic line of descent of their cells. The ribosomal proteins changed considerably but if a primitive cell hand a ribosomal RNA similar to that of its modern descendants the overall molecular weight of its ribosomes must also have been of the same order of magnitude, whatever the characteristics of the ribosomal proteins. I express this by saying that the phylogenetic ancestors of cells which now possess 70S and 80S ribosomes had to have 70S-like and 80S-like ribosomes.

(2) High molecular weight ribosomes had to be present at a very early stage of cellular evolution because without them translation would be inaccurate and biological specificity would be low. More precisely, if a fossil record shows the remnants of specialized cellular structures we are bound to conclude that those cells possess high molecular weight ribosomes.

(3) There must have been a phase, during the early history of life, when ancestral ribosomes evolved from low to high molecular weight prototypes.

It will be noticed that these statements do not contain any reference to the specific progenote theory proposed by Woese. They represent, in my opinion, the lasting part of Woese's contribution while his progenote theory may be criticized and abandoned. The model that I propose does precisely that, but I hope that my criticism of Woese's specific model will not obscure the fact that I am borrowing heavily from him the basic concepts that I have listed above.

The main thesis of my model is that the evolution from low to high molecular weight ribosomes took place during precellular evolution and

was caused by the natural selection of quasi-replicating systems of increasing complexity; once the protocells were formed, however, the molecular weights of their ribosomes could either remain stationary or evolve downwards.

Let me briefly summarize the difficulties that an upwards evolution of the ribosome molecular weight encounters if it is carried on within a cellular framework.

Woese himself has pointed out that a cell with low molecular weight ribosomes would have a very low degree of biological specificity and any gene would be translated into a group of statistical proteins. I add that such a cell would soon "learn" that any of these statistical proteins could be synthetized from a slightly different gene and would end up with "statistical genes" as well as statistical proteins. The spectrum of any gene-to-protein group correspondence would increase instead of decreasing and when the spectra of different genes or proteins overlap the cell would face total chaos.

Let us assume, however, that a cell managed nevertheless to increase the molecular weight of its ribosome and to achieve the 70S-like type of ribosomes which guarantee the highest degree of translation accuracy and a one-to-one correspondence between genes and proteins. Any mutation which further increases the molecular weight of such ribosomes would not increase their translation accuracy but would surely increase the metabolic burden of the cell and would therefore be selected against. Even if all mutations which transform 70S into 80S ribosomes were to occur at once the cell would still be unable to benefit from them unless we admit that all the other essential eukaryotic characteristics came into existence simultaneously.

I conclude that 70S-like ribosomes cannot evolve into 80S-like ribosomes within a cellular system and incidentally this is my reason for concluding that prokaryotes could not give origin to eukaryotes. More generally, I conclude that within a cellular framework the molecular weight of the ribosomes cannot evolve upwards. The protocells had therefore ribosomes of the 80S type or heavier.

I have already mentioned that the evolution of heavy ribonucleoproteins took place at a precellular level and it is possible to rationalize the driving mechanism which was behind it. If the ribotype hypothesis is correct a mature ribosome is the final product of a specific biogenetic process and we cannot have, for example, 70S ribosomes from an 80S type of ribosome precursors or an 80S type of ribosome biogenesis. 70S ribosomes are good enough for translation but their precursors form very poor supramolecular aggregates and these are not good enough for quasi-replication.

At the precellular level natural selection was working not only on the translation properties of the ribosomes but also, and perhaps predominantly, on their biogenetic properties and favoured 80S-like ribosomes because they were associated with supramolecular aggregates of ribosome precursors which were better suited for quasi-replication purposes. Their three-dimensional networks could grow bigger and they could provide structural and functional centres of biological activity for the development of quasi-replicating systems of increasing complexity.

There is, therefore, a rational explanation for the conclusion that the protocells contained 80S-like ribosomes and in the next section I will add another argument in favour of this conclusion by showing that it provides a most natural interpretation of the events which followed the origin of the protocells.

First, however, I will complete the present analysis by pointing out that Woese's molecular genealogy justifies one more conclusion. If the protocells had 80S-like ribosomes they were the phylogenetic precursors of today's eukaryotes.

It is essential to notice that the protocells had almost none of the characteristics of the modern eukaryotes: large dimensions, mitochondria, chloroplasts, mitosis, etc., were all absent at such an early stage. And yet their nucleoli-like cores and the 80S-like characteristics of their ribonucleoproteins are sure indications of their phylogenetic link with the future eukaryotes and for this reason I will call them "microkaryotes".

2(G) THE EVOLUTION OF THE PROTOCELLS

One of the major conclusions of the ribotype theory is that the first cells which appeared on earth had 80S-like ribonucleoproteins and were, therefore, the phylogenetic ancestors of the eukaryotes, even if they lacked almost all the characteristics that we now associate with the eukaryotic cell.

In this section it will be shown that this conclusion provides a natural explanation for the splitting of the primeval cells into the two major phylogenetic groups which became the ancestors of today's prokaryotes and eukaryotes. Once the microkaryotes were formed any mutation which could reduce the amount of energy and material resources required for the production of the ribosomes without impairing their translation accuracy would give origin to less demanding and more efficient cells and would, therefore, be strongly favoured by natural selection.

The 80S-like ribotypes of the microkaryotes could be streamlined in a variety of ways; ribosome biogenesis could be simplified and various genes for ribosomal proteins could be eliminated altogether while others could

be reduced in size and code for smaller proteins. We can well imagine that Nature tried all sorts of experiments in this direction and that only a fraction of them proved successful. It is likely, for example, that ribosomes with molecular weights even lower than those of the prokaryotes were tried but these would not have been able to ensure a high degree of biological specificity and were therefore selected against. The evolution towards low molecular weight ribosomes had a natural limit in its need to preserve a high level of translation accuracy and 70S-like ribotypes emerged as the best compromise.

In a primitive environment the advantages of a prokaryotic cell organization were manifold and it is not surprising that prokaryotes could flourish spread and differentiate in the variety of types which colonized the earth.

The ribotype theory has therefore no problem in accounting for the age of the prokaryotes which is documented by the fossil records. A problem might instead arise with the survival of the emerging eukaryotes. If the tendency of the microkaryotes to become prokaryotes was strongly favoured and if the evolution from high to low molecular weight ribosomes was practically irreversible, it may appear that the prokaryotes were destined to become the sole inhabitants of the earth.

Such a conclusion, however, is by no means inevitable. In fact it would be unreasonable to assume that the transformation of 80S-like into 70S-like ribonucleoproteins was the only evolutionary course open to the microkaryotes. Various mutations could have improved the overall efficiency of the cell without changing its ribotype and among all mutations which affected the ribonucleoproteins many could have been beneficial to the cell economy even if they did not alter their 80S-like nature.

The ribotype of the microkaryotes had evolved on the basis of quasi-replication strategies and it is only natural to assume that it could be further adapted to a proper replication strategy without undergoing a massive dismantling of its original structure. Furthermore, even if the prokaryotes were the most efficient types of primitive cells they did not represent an immediate threat for the other types because at the beginning of cellular evolution the population density of the cells was low and the resources were ample. A fierce competition among the cells was bound to become real only at a later stage and it is reasonable therefore to conclude that microkaryotes with 80S-like ribotypes could survive and evolve for a considerable interval of time.

I summarise the process by saying that some microkaryotes became microeukaryotes and others became prokaryotes. On energy-competition grounds the microeukaryotes were at a disadvantage in respect to the prokaryotes but they were also more complex types of cells and as long as

they could produce descendants the possibility to take advantage of their greater potentials remained intact.

It will be noticed that we are approaching here the same situation as that described by Stanier when he concluded that a conflict had to originate between the flexible prokaryotes and the more complex cells of the emerging eukaryotes. The endocytosis model has already provided a most convincing and elegant solution for this eventuality and I do not hesitate to follow Stanier's approach. The ribotype theory differs therefore from the other models only in respect to the reconstruction of the precellular and protocellular phases of evolution as shown in the diagram of Fig. 1. As far as proper cellular evolution is concerned, the theory is compatible with solutions already proposed by other authors and can be integrated without conflict with existing theories.

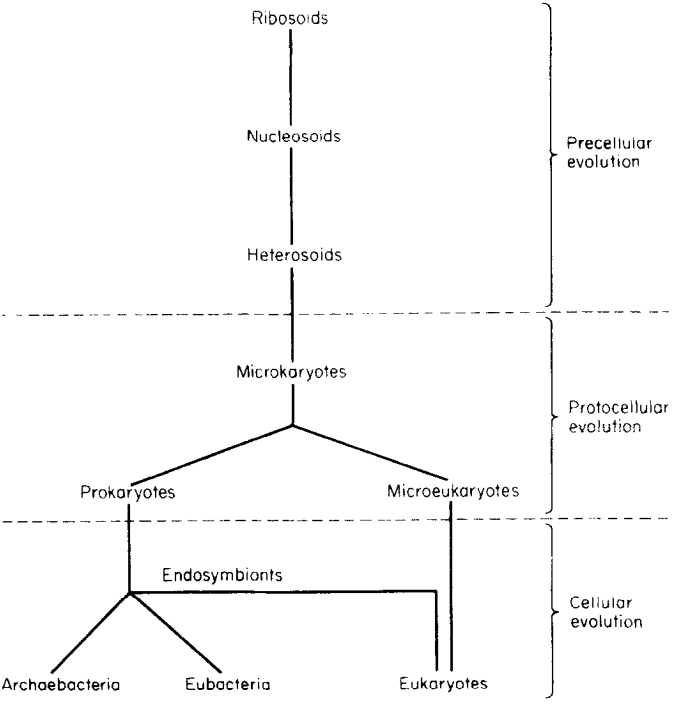

FIG. 1.

3. A New Natural Philosophy

3(A) THE CELL PROBLEM

During the last century and the first half of the present one, proteins were regarded as the fundamental components of the cells and correspondingly it was believed that life started with coacervates of primitive proteins. The discovery that DNA is the basis of heredity has given a sort of conceptual priority to the genes and a new orthodoxy has proclaimed thereafter that life started from naked genes.

It is clear from these examples that a theory on the origin of life is essentially a conceptual mirror of what are the primary components of life for one or more generations of biologists. More precisely any theory on the origin of life is a reflection of whatever belief is held on the essence of the cell, the logic being that if we understand what a cell ultimately is we would be a long way on the road to understanding how it could have originated.

The development of the ribotype theory differs from that of the previous ones because it is not the reflection of a widely diffused belief on the basic components of life. Historically we are still very much in a period of DNA supremacy and it will take perhaps a new generation of biologists to realize that genes alone could not have started life on earth any more than proteins alone could.

The reason for this is that we are imbued with the concept that a cell is essentially a throwaway survival machine built by the genes, and a genuinely new attitude toward the origin of life will become popular only when this view is replaced by a different one. In this chapter I want to show that the framework for such an alternative theory already exists.

The ribotype reconstruction of precellular and protocellular evolution has been described in the previous chapters with an apparently arbitrary sequence of hypotheses but now I want to show that behind them there is a unifying motive and that this leads naturally to a new theory on the nature of the cell.

3(B) THE RIBOTYPE HYPOTHESIS (GENERAL VERSION)

The conventional view of the relationship between the cell and its ribonucleoproteins can be summarized as follows. Natural selection works on phenotypes and the ribonucleoproteins adapted during evolution to the role of translation instruments of the cell. Even if one accepts the ribotype view that it was the ribonucleoproteins which created the cell, it is natural to conclude that that was just precellular history. From the protocells

onwards the essence of ribonucleoprotein evolution was their adaptation to the role of linkages between genotype and phenotype. This conclusion is supported by no evidence but is nevertheless a very strong belief because it is a necessary consequence of the genotype–phenotype paradigm. I propose to leave it aside for a moment and to examine what happened during evolution from a different angle.

Let us start with the prokaryotes. In my view the transformation of an 80S-like into a 70S-like ribotype was not an ordinary event by which the emerging prokaryotes acquired just one of their various characteristics. It was instead *the* event which made of a cell an obligate prokaryote, and the acquisition of all the other prokaryotic characteristics followed as subordinate readjustments.

Let us examine what a cell with a 70S ribotype could do. It could not decrease further the molecular weight of its ribosomes because it would have lost translation accuracy and biological specificity. It could not reverse the process and acquire again heavier ribosomes because the process was virtually irreversible. There was no alternative but to keep a 70S type of ribosomes and with it a 70S type of ribosome biogenesis which is incompatible with an intracellular segregation of the genome. The cell was trapped into the prokaryotic organization. The only evolutionary course which was open to the primitive prokaryotes was to make the best of a cellular organization where the genes have to be exposed to the cytoplasm, and the ideal limit of such a course is probably the organization which was historically developed: a cell type where transcription is physically linked to translation and the regulation of protein synthesis is based on short-lived messengers.

As for the eukaryotes they had, in principle, an additional degree of freedom because the ribosome molecular weights can evolve downwards and the eukaryotes could therefore have become prokaryotes at all stages of evolution. But the sequence homology evidence indicates that they did not. The transformation of 80S-like into 70S-like ribotypes occurred once at a very early phase of evolution and was never repeated afterwards.

I conclude that the transition from microkaryotes to microeukaryotes was a qualitative development which transformed the ancestral 80S-like ribotypes so deeply as to make any future transition to the 70S type virtually impracticable. Another equivalent possibility is that the microkaryotes had a heavier ribotype—let us call it a 90S type—which could evolve downwards toward an 80S or a 70S type with no further possibility of converting any one of these ribotypes into the other. Even the microeukaryotic cell was therefore trapped into a definitive framework and could only evolve along a developmental path which was compatible with its ribotype.

An 80S ribotype implies the existence of a nucleolar-like supramolecular matrix in the region of the genome which makes it difficult, if not impossible, for all the genes to be directly accessible to the ribosomes for the immediate translation of their messengers. Some sort of physical separation between transcription and translation had therefore to exist in the microeukaryotes long before the development of the nuclear membrane and the regulation of protein synthesis was bound to evolve on the basis of long-lived messengers.

I come therefore to two conclusions on the history of the cells. The first is that the nature of the 70S and 80S ribotypes of the primitive prokaryotes and microeukaryotes did not change much after protocellular evolution. This conclusion is based primarily on the evidence provided by the sequence homology data but is also supported by the characteristics of chloroplasts and mitochondria. They show that prokaryotes and eukaryotes lived in symbiosis for more than a billion years without mixing their ribotypes and that the ribotypes of the prokaryotes which lived in symbiosis did not change much in respect to those of the free living bacteria. The second conclusion is that the divergence from the common ancestor left the primitive prokaryotes and microeukaryotes with ribotypes which constrained their cells to follow two opposite evolutionary courses in respect to the relationship between transcription and translation and, correspondingly, in respect to the regulation mechanisms of protein synthesis.

With these conclusions we can now re-examine the statement that the essence of ribonucleoprotein evolution was their adaptation to the role of linkages between genotype and phenotype. This statement is not wrong, it only covers a very small part of the truth. The rest of the truth is that the ribotypes could adapt very little and it was therefore the cell that had to adapt to them if an integrated cellular system was to develop at all.

We can look at the relationship between cell-type and ribotype from two different viewpoints. One is the idea that the cell is a genotype–phenotype entity which is largely independent of its ribotype in the sense that it could have used for translation unlimited varieties of ribonucleoproteins if only these had been available. It so happened, however, that the translation machines of the first cells could not be substituted and natural selection was only able to favour minor readjustments of their original designs.

My view is that such a concept of an independent cellular essence is a fiction. It gives a conceptual priority to the cell as if it was a platonic idea existing before the real world without explaining how it could have originated. Furthermore, it implies that the cells are still using primitive

translation machines which absorb a disproportionate amount of their resources because there was no better substitute for them in nature.

In my opinion, what we call a cell is simply what managed to integrate with the primitive ribotypes. The structures and functions which emerged and were compatible with the existing ribonucleoprotein systems could survive, the others were eliminated. There is a perfect integration between the cell and its ribonucleoproteins and if the ribotypes could not change much it was the rest of the cell which had to be moulded on them.

I summarize these concepts with the aphorism: "One ribotype, one cell-type". This represents the generalized version of the ribotype hypothesis, while the restricted version was correlating the ribotype with ribosome biogenesis only.

What therefore is the essence of the cell for the ribotype theory? The cell is a colony of ribonucleoproteins engaged in producing other colonies of ribonucleoproteins. There was no real discontinuity between precellular and cellular evolution. Only the acquisition of sophisticated replication mechanisms brought about by the evolution of the quasi-replication mechanisms which had been developed by the ancestral ribosoids to produce other ribosoids. The fact that the cell invests much more of its energy and material resources in its ribotype than in its genotype indicates in my opinion the real purpose to which the cell economy is orientated. The fact that the ribonucleoproteins did not substantially change during evolution and represent the only survivors of the ancestral forms of life is yet another indication of what are the real invariants which life has to preserve in order to perpetuate itself. The ribonucleoproteins created the cells in the first place, and are still using them to replicate themselves.

3(C) SUBCLASSES AND SUBTYPES

The general version of the ribotype hypothesis states that a 70S or an 80S ribotype is a precondition for the development of a prokaryotic or an eukaryotic cell organization. It will be noticed that there is no causal relationship between ribotype and cell-type. The ribotype does not provide instructions but only constraints and means for the development of the cell-type.

The absence of a deterministic linkage implies that the relationship between ribotypes and cells is a broad class-to-class correspondence, but this is not incompatible with the existence of subclasses within each class. For example, when chloroplasts and mitochondria lost their cellular status and became endosymbionts they became a special subclass of cells and the ribotype hypothesis predicts that they must have a special subclass of

ribotypes. There are in fact indications that the ribosomes of chloroplasts and mitochondria are in a class of their own even if their prokaryotic ancestry implies that they are close relatives of the bacterial ribosomes. Another example of subclasses of cells which arise in correspondence with subclasses of ribotypes comes from the division of the prokaryotic kingdom in eubacteria and archaebacteria. Woese has discovered the existence of this phylogenetic separation by studying the sequence homologies between the ribosomal RNAs of the two groups of cells, and from the point of view of the ribotype theory this is no coincidence. A difference among ribotypes would necessarily result in a difference of cell-types.

At this point we may be tempted to extend the ribotype hypothesis even further and conclude that the minor differences which exist among ribonucleoproteins of different species were also the cause of evolutionary differences among their cell-types. Here however we must exercise some caution. If the relationship between ribotypes and cells was a deterministic one the conclusion would be legitimate, but the ribotype hypothesis does not state that. The one-way correspondence from ribotypes to cell-types is valid only in the sense that the cell cannot change the nature of its ribotype and can evolve exclusively within the organizational limits which are compatible with its ribotype. The reverse correspondence from cell-types to ribotypes has therefore a limited range of possibilities but the theory does not forbid it, and minor variations of the ribotypes which amount to adaptations to their cell-types could also have taken place. The relationship between ribotypes and cell-types is therefore a complex one and at present we can only grasp its basic outlines.

A more detailed description will have necessarily to wait for the discovery of the functions of the individual components of the ribosomes and of the ribosome precursors which at present are still largely unknown.

3(D) THE REALITY OF THE RIBOTYPE

All genes have physical structures, but as three-dimensional molecules different genes are virtually identical; they wind up in space and unwind for replication in the same way. As a first approximation at least we can say that differences among genes do not exist in the three-dimensional world, and in this sense genes are real only in the one-dimensional world of linear information. For proteins, the reverse is true. A protein cannot unwind and pass on information about the linear sequence of its own aminoacids and it is only a specific three-dimensional structure that gives a protein its biological function.

The real distinction between genotype and phenotype is based therefore on the distinction between the one-dimensional world of information and the three-dimensional world of physical structures. The critical point is that there is no *direct* communication between these two dimensions of reality. A gene cannot build a protein any more than a protein can instruct a gene.

The central dogma states that information does flow from genes to proteins, but only because it has been taken for granted that a third party exists which can actually implement the transition. What is not usually emphasised is that such an intermediary cannot be either another group of genes or another group of proteins. The reality of the ribotype comes in not when we realize that an intermediary between genes and proteins is essential, but when we realize that such an intermediary has characteristics which are neither those of the genes nor those of the proteins.

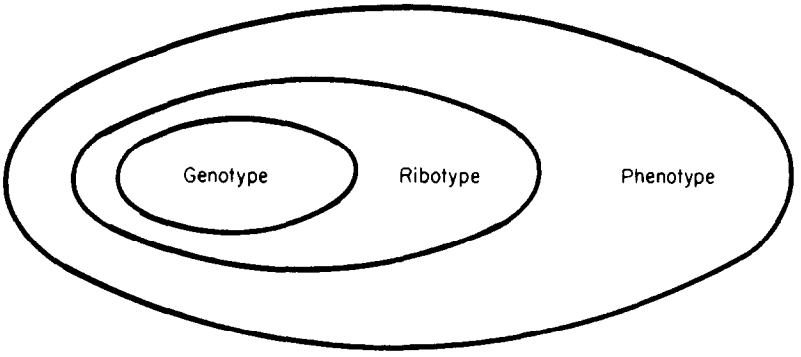

FIG. 2.

There are ribosoids which carry linear sequences of instructions and others which wind up in space and perform functions in the three-dimensional world of the physical structures. It is only because one-dimensional ribosoids and three-dimensional ribosoids are able to interact and integrate that information can flow from the one-dimensional to the three-dimensional world. If only genes and proteins were to exist, the bridge could not be crossed, and we are bound to conclude that every organism is based on a tripartite reality whose material substrates are genes, ribosoids and proteins (Fig. 2).

It will be noticed that the above argument was based on general knowledge only, and the concept of the ribotype could and should have been introduced into biology a long time ago.

The same conclusion can be reached in another way. The definition of an organism as a duality of genotype and phenotype implies first that the phenotype is the expression of the genotype and, second, that the ribotype is part of the phenotype, but both conclusions are inaccurate, to say the least. The history, the etymology and the common use of the term, all indicate that the phenotype is the phenomenological living being. It is the organism as it appears from the outside world, the sum of its interactions with the environment and with the other organisms. This implies that the phenotype cannot be identified with all that is expressed by the genotype but only with part of it, and precisely with that part which results in phenomenological relationships.

As an extreme example let us consider an hypothetical exchange of ribosomal genes among the cells of different species. Within the framework of classic biology this would not affect the efficiency of the translation apparatus, and cells could therefore have different ribotypes with the same phenotype. The same conclusion can be obtained by considering, more realistically, that mutations could change the ribotype without affecting the phenotype, and the assumption that the ribotype is part of the phenotype is clearly inconsistent.

The very definition of phenotype leads us therefore to conclude that the genotype–phenotype duality cannot be a complete theoretical description of an organism. It is a didactic concept which was introduced by Johannsen in 1909 to differentiate between hereditary and phenomenological characteristics, and it was only an unfortunate accident that the duality has been elevated to the status of a theoretical category.

The ribotype theory, however, goes a bit further. The theory not only recognises that the ribotype is as fundamental as genotype and phenotype are, but attributes to it an evolutionary priority. Since ribosoids can carry linear instructions and exhibit a wide variety of three-dimensional structures, the ancestral ribotypes had all that is required for the transfer and the expression of biological information. They contained, in other words, what can be described as their own ribogenotype and their own ribophenotype.

Using this terminology, the evolution towards cell organization can be summarized by saying that the cell genotype evolved as the extension of the ribogenotype, and the cell phenotype as the parallel extension of the ribophenotype (Fig. 3). Today we often consider only the ends of the trinity, but without their necessary intermediary we could not understand how

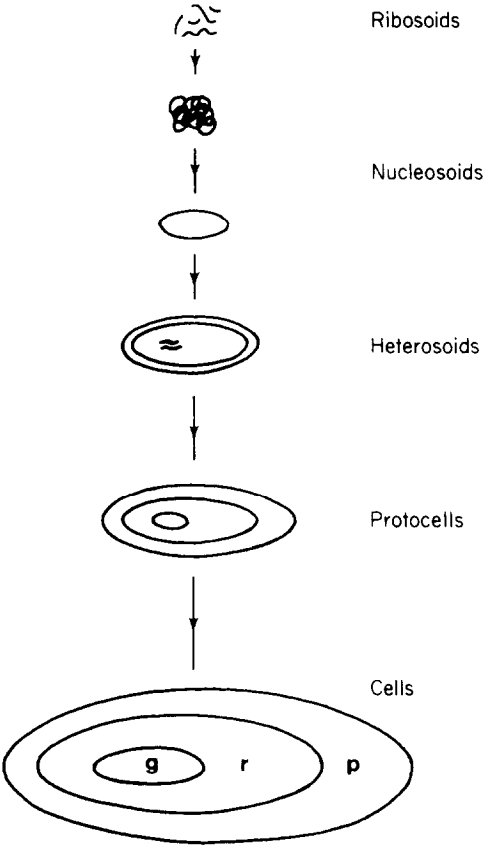

FIG. 3.

they form a functional unity. The ribotype theory adds that we also could not understand how they originated in the first place.

3(E) THE PHENOTYPE THEORY

The phenotype theory states that life started with droplets or coacervates of primitive proteins, and its main proponent has been, since 1936, Alexander Oparin. Oparin has no shortage of followers in the West (J. D. Bernal and S. W. Fox, for example, are among them) but it was Soviet Russia and more precisely the New Soviet Biology dominated by Lysenko which provided the fertile background for the development of Oparin's view. The reason is straightforward enough. Lysenko's biology is a form of Lamarck-

ism and Oparin's theory is nothing but molecular Lamarckism applied to the origin of life. If primordial metabolizing systems devoid of a genetic programme were the precursors of the first cells, we have to conclude that somehow biological information was being transferred from protein to protein and later from proteins to genes, which are precisely the two faces of Lamarckism at the molecular level.

But how was it that Lysenko's biology could easily be rejected whereas Oparin's views were not!? The answer lies in what can be described as the fundamental axiom of the phenotype theory: the origin of life must have preceded the origin of heredity. The first protein droplets had to produce descendants and had therefore to acquire, somehow, the ability to perform some sort of replication. The axiom implies, as a consequence, that replication preceded heredity, and this would be perfectly legitimate if we could associate heredity only with DNA and replication only with proteins.

Protein droplets could, of course, originate spontaneously; they could even grow by the accumulation of materials and eventually break down into smaller pieces, but this is not what replication is about. A biological form of replication cannot depend exclusively on passive processes of accumulation and segregation but requires, sooner or later, the ability to perform the active synthesis of the materials which are to be distributed among the descendants. It is true that we can leave DNA out of this, but the phenotype axiom breaks down because we cannot leave out RNA. More precisely, the phenotype theory would be a valid approach if only it were possible to devise a mechanism for protein synthesis which relied exclusively on proteins.

It is not difficult to see the historical reasons which are behind the phenotype theory. Before 1944 proteins were regarded as the substrate of heredity, and as long as biological information as well as biological structures were thought to be expressed by proteins, there simply was no other alternative but to conclude that life started with the first proteins which appeared on earth. The discovery of DNA's function by Avery and his colleagues changed the situation, but not completely—because DNA is not involved in protein synthesis. The idea that some form of replication which is not based on DNA preceded the formation of the first cells was, and still is, perfectly legitimate. But then we realize that this form of replication had to rely on ribosoids and this leads us naturally away from the phenotype theory and towards the ribotype solution.

3(F) THE GENOTYPE THEORY

The DNA revolution has brought us also, together with genetic engineering and sociobiology (Wilson, 1975) a new theory on the origin of life.

This theory is the evolutionary projection of the idea that organisms are simply DNA's way of producing more DNA, but, as Doolittle & Sapienza (1980) have remarked, this statement "has been made so often that it is hard to remember who made it first". I will therefore leave its paternity problem to the historians and only add that my own sources of information have been the third edition of the "Theory of Evolution" by John Maynard Smith (1975) and the delightful "Selfish Gene" by Richard Dawkins (1976).

The genotype theory states that life started from naked genes and not from proteins for the very good reason that genes replicate while proteins do not. According to this theory a variety of compounds originated spontaneously in the primeval solutions and the molecules of DNA which appeared among them began to produce copies of themselves. The fact that today their replication requires specific enzymes is not a great obstacle: similar enzymes could also have originated spontaneously or simpler catalysts could have been used at the initial stages. The replication mechanism, of course, was not perfectly accurate, and occasionally errors would have occurred which introduced a very important degree of heterogeneity into the growing population of replicating molecules. Eventually, however, the process was bound to come to an end because the natural supply of nucleotides was not unlimited. What happened then in the primeval solution?

We could compare the polymerization of nucleotides to the formation of crystals in a saturated solution and conclude that when equilibrium was reached the process, macroscopically, would stop. But the genotype theory describes quite a different scenario. It states that in a situation of scarce resources the replicating molecules started competing among themselves for the few available nucleotides. "Some of them may even have discovered how to break up molecules of rival varieties chemically, and to use the building blocks so released for making their own copies. These proto-carnivors simultaneously obtained food and removed competing rivals. Other replicators perhaps discovered how to protect themselves, either chemically, or by building a physical wall of protein around themselves. This may have been how the first living cells appeared." (R. Dawkins, 1976, p. 20.)

What is wrong with this description? If, instead of speaking of replicating molecules, we were speaking of dinosaurs, birds, ants and microbes, the whole thing would make sense. It is a classic struggle-for-life scenario in straightforward Darwinian terms and I better add that I am a Darwinist. The problem is that the traditional struggles for life used to make sense only from the cellular level upwards, while the genotype theory is claiming the ultimate generalization of Darwinism by extending it to the molecular

world. There is, however, a classic example which should suggest restraint.

We can study the properties of water with a bucket, a drop or even a molecule of it, but as soon as we break the molecule the water does not exist any more. Let us be a bit more specific. For example, how could replicating molecules of DNA surround themselves with membranes? They could find membrane proteins readily available in solution but their replication would require a transfer of information from proteins to genes. Alternatively, a DNA molecule could, by chance, arise with precisely the right sequence of nucleotides which code for a membrane protein, but in order to synthetize it with even a low degree of accuracy, it would need the translation apparatus of a primitive cell. A cell would have to exist in order to build the components which make up the cell in the first place.

At this point, one could suggest that the analogy between the cell and the molecule of water can be used against the ribotype theory as I have used it against the genotype theory, but such a proposal would not be consistent. A cell is essential for self-replication not for quasi-replication. A strategy based on DNA requires carbon copy mechanisms which produce exact molecular replicas of the progenitors in their descendants, whatever the degree of noise in the real apparatus, and it is therefore inevitably a strategy of self-replication.

The ribotype theory, on the other hand, has based precellular evolution on a quasi-replication strategy which can operate at the level of supramolecular aggregates. The important point is that the theory has not invoked hypothetical new properties for such a strategy but has only relied on well known characteristics like polymorphism, self-assembly and translation ability of the ribosoids. Furthermore, the theory has shown that quasi-replicating systems can evolve towards higher levels of complexity and has drawn the consistent conclusion that the first cells appeared precisely when quasi-replication evolved into self-replication.

If we base the origin of life on DNA we are therefore bound to assume that a complete self-replicating unity of genotype and phenotype came suddenly and spontaneously into existence. If we assume, instead, that self-replicating systems evolved gradually from pre-existing forms of life then we have to abandon DNA as their basic substrate and turn to the ribosoids. The paradoxes of the genotype theory lead us naturally to the ribotype solution as those of the phenotype theory had done.

3(G) THE ROLE OF DNA

The sociobiologists have been outspoken supporters of the genotype theory but they have also entertained the idea that naked genes were not,

after all, at the origin of life. The important point is that whatever started life on earth soon fell under the total control of DNA. In "The Selfish Gene" Richard Dawkins expressed this concept admirably: "Usurper or not, DNA is in undisputed charge today".

The problem is that many biologists would instinctively agree with this statement, and we should therefore take a closer look at it. In which sense can we say that DNA is a molecular usurper which took charge of the cell? The first image which comes to mind is that of DNA molecules which invaded the primitive replicating systems and took control of them, very much like viruses which attack a cell and force it to produce their own components.

Is this analogy a proper one? It could be, if we imagine that the DNA invaders and the other molecules which were using nucleotides started competing for them and DNA eventually won the contest. As I said before, I do not believe in these molecular struggles, but since they are a favourite theme of the sociobiologists they should meditate upon the fact that if such a struggle ever took place, DNA came out the loser, not the winner. The majority of the cell nucleotides are devoted to the production of ribosoids, not of genes.

In my opinion it is not the analogy of the invading usurper which provides the best description for the role of DNA, but the analogy of the parasite. A successful parasite would never drag out of its host more than a fraction of its resources, otherwise it would risk producing their mutual destruction. Furthermore, a successful parasite would have to do something useful in return, otherwise the host might find it convenient to dispose of it. These two characteristics fit remarkably well with the experimental pattern. The genes absorb only a fraction of the cell nucleotides and make themselves useful by providing highly stable substrates for the master copies of the biological instructions. It is then natural to conclude that the cell adapted the replication mechanisms of its master copies to the specific properties of DNA and eventually became irreversibly DNA-dependent. The parasite had secured its survival for good by becoming an essential component of the cell machinery.

Strangely enough, the idea that some genes behave as molecular parasites was proposed by Dawkins himself, and was later developed extensively by Doolittle & Sapienza (1980) and by Orgel & Crick (1980). The problem that these authors were confronting was the paradox that large amounts of DNA in most organisms seem to have no phenotypic value and represent an inexplicable waste of repetitive sequences. They explained the paradox by showing that a genome which contains specific, or useful, DNA provides a fertile ground where a second class of DNA can originate, spread and

replicate even if it is of no use to the cell. No other explanation for its existence is required other than its natural tendency to parasitize other genes in order to ensure its own survival within the genome.

This second class of DNA was referred to as "selfish DNA" and Orgel and Crick labelled the selfish genes as "the ultimate parasites". I do not hesitate to accept this solution. It is simple, elegant, logical, persuasive. But why go only half way? If the selfish genes originated through a parasite-like mechanism why should not the same be true for the others? The selfish genes were the parasites of the useful genes as these had been the parasites of previous RNA-genes. This is the ribotype solution. With it, all DNA molecules acquire the same status of molecular parasites and a unitary mechanism is used to explain their origin and evolution.

Let us now return to the statement that "DNA is in undisputed charge today". Most biologists would instinctively agree with it because they would assume that it is simply a new version of the well-established concept that DNA is the carrier of hereditary information. But the sociobiologists have much more in mind. Their meaning is that the cell economy is entirely oriented to the reproduction of DNA and every life process is subordinated to this primary goal. That this is their true perception of life is demonstrated by their definition of any living being as a "throwaway survival machine" built and used by the genes as a disposable container.

It is this second interpretation that I object to. If DNA is a molecular parasite then we cannot say that DNA is in charge of the cell any more than a parasite is in charge of its host. Nor can we say that DNA was at the basis of the origin and evolution of life any more than we can say that the evolution of a parasite preceded and determined that of its host.

The ribotype theory puts the ribotype metabolism at the basis of the cell economy in the same sense that we attribute to the metabolism of a host a conceptual and an evolutionary priority over that of its parasite without underestimating the fact that the parasite has long since become an essential part of the system. The genotype theory and sociobiology have put DNA at the centre of life as the earth was at the centre of the Ptolemaic universe. The ribotype theory removes DNA from such a privileged position but it is only the mythology of DNA that we lose, not the perception of its real role in Nature.

3(H) A REVIEW OF THE ALTERNATIVES

In addition to the theories described in the previous chapters there are three other hypotheses on the origin of life which should be briefly mentioned. The first two are the creationist view and the idea that life was

brought to earth from other planets (Panspermia). The third is the spontaneous generation of the first cells from the molecules of the primitive solutions, a view which is the last surviving version of the old belief that small organisms could suddenly and spontaneously originate from decaying organic matter. These three views are not going to be discussed here because they raise philosophical, historical and religious problems where the emphasis is on personal belief and could be addressed properly only in a different essay.

If we accept that the primitive cells did not appear suddenly on earth but emerged from a long series of evolutionary developments, we have therefore to choose between the three views which I have referred to as the phenotype, genotype and ribotype theories. In general, this conclusion is not accepted easily because there is a widespread feeling that things might have happened in an enormous variety of different ways, but here we have to distinguish carefully between principles and details. The actual details with which any of the three basic theories can be implemented form a truly formidable class of possibilities, but this should not obscure the fact that the underlying principles are, in reality, very few.

The three theories which I have described can therefore be formulated with a great variety of different specific mechanisms, but any conceivable scientific model is bound to be a version of one of them or a combination of their different principles, unless there is a revolution in our basic understanding of Life and Nature. Given this situation, it is important to see if our three classes of scientific models on the origin of life are equivalent or not. Are they simply different reference systems which allow us to look at the same body of experimental data from different but equally legitimate points of view?

If we consider, for example, the theories on the solar system, we might well prefer the Copernican view, but we know that the Ptolemaic system was also capable of describing the motion of the planets with accuracy. Conceptually, we could use either system and we could end up with charts which tell us exactly where each planet is going to be in respect to the earth at any given time. In this sense the two systems are equivalent and we are interested here in discussing if a similar sort of equivalence exists for the three basic theories that have been proposed on the origin of life.

The answer is no. There is a fundamental difference between a theory which makes use only of concepts for which we have positive evidence and a theory which has to rely on processes which violate our present understanding of nature.

The phenotype theory, for example, is based on a transfer of biological information from protein to proteins and from proteins to genes which is

in contradiction with what is taking place now. We cannot say that it did not happen, or that it could not have happened that way. What we are entitled to say, however, is that the phenotype theory relies on basic differences between past and present laws of Nature. The same is true for the genotype theory. The idea that during precellular evolution the primordial genes could promote the direct synthesis of the components which were instrumental for their replication is equally in contrast with what is taking place now, even if the fault is more difficult to spot because the direction in which information flows is, in this case, the right one.

The ribotype theory, on the other hand, is based only on well-established concepts. One might object that this is not true because the idea of quasi-replication, to give just one example, is a novelty, but the objection misses the mark. The important point is that the concept of quasi-replication is based exclusively on properties like polymorphism and self-assembly, for which we have strong positive experimental evidence. It is true that there are new ideas and hypothetical features in the ribotype theory, but it is crucial to notice that they have been built on existing natural properties and that they rely on a continuity between past and present laws of Nature. This is not enough to prove the theory right, but it is sufficient to conclude that the theory is not exactly equivalent to its rivals.

3(I) A THEORY FOR THE FUTURE

Any theory in a speculative form, like the one described here, is bound to be regarded with suspicion, if not with contempt. This is perhaps inevitable, but I will nevertheless try to defend the approach that I have chosen. I admit that even a sympathetic biologist is likely to accept the statements in the ribotype theory with a great many reservations. Many, for example, would basically agree with the idea that eukaryotes cannot utilize 70S ribosomes, but would hastily add that we cannot be sure.

Given this situation would it not be more sensible to wait until all the necessary evidence is collected? The answer is no. Such an attitude is based on the assumption that scientific progress takes place as an inexorable accumulation of data on all fronts, and that theories are there only to make an inventory of what has been discovered. This is therefore my first justification. A theory was necessary before all the evidence is collected in order to stimulate the collection of data which will eventually provide the necessary evidence. A theory can legitimately stand only on its own internal consistency.

The statement that eukaryotes cannot utilize 70S ribosomes, for example, is perfectly legitimate within the framework of a consistent synthesis. If

anyone has doubts about it, he is entitled to do experiments and try to prove it false, but not to conclude that a theory cannot make use of such statements until there is conclusive proof that they are right. Karl Popper (1959) illustrated this point with admirable clarity and there is no need for me to elaborate further.

The second reason for my approach came from outright uneasiness about the ideas which surround the studies on the origin of life. This is the only field where Lamarckism is still taken seriously and where there seems to be nothing paradoxical about chicken-and-egg paradoxes as long as they are confined to the molecular level.

I believe that what is now known is enough to draw positive conclusions about some of the crucial events which happened at the beginning of life if it is used with rigorous logic.

For example, let us take Woese's idea that high-molecular weight ribosomes are a precondition for biological specificity. This is a conceptual milestone. It gives us the theoretical certainty that high-molecular weight ribosomes had to exist at the very beginning of cellular evolution, whatever else was going on. One is obviously entitled to doubt it, but the only thing to do in this case is to set up appropriate tests. The study of ribosomal mutants, the reconstitution *in vitro* of ribosomes with artificially altered components and the selective engineering of ribosomal genes are all means which can evaluate the effect of significant alterations of the ribosome molecular weights.

In the meantime we can legitimately assume that the idea is true simply because there is no cell which can use low molecular weight ribosomes, and then go on to the next logical step. If high molecular weight ribosomes had to exist at a very early phase of the history of life their evolution from low molecular weight particles could have taken place either within primitive cells, as Woese himself suggested in the progenote theory, or at a precellular level.

But the progenote theory leads us inevitably into paradoxes. How could a progenotic cell replicate its own ribosomes and itself if even the simplest form of self-replication requires a degree of biological specificity which is ensured only by high molecular weight ribosomes? How could a cell which produces statistical proteins avoid producing also statistical genes and evolve towards increasing order if it is programmed to produce increasing disorder?

When all this is translated into the rigorous language of information theory and bioenergetics biologists will realize that we are left with only one alternative: the evolution from low to high molecular weight ribosomes had to take place at a precellular level. This will no longer be an arbitrary

assumption of the ribotype theory. It will be a conceptual necessity for every rational mind.

The third reason behind this essay is an old-fashioned distaste for accidents applied to a particular case. All prokaryotes have 70S ribosomes, and all eukaryotes 80S ribosomes. Within the framework of today's biology this simple, solid, universal fact is an accident. Accidents of course do happen, but not of this magnitude. When they affect all living beings and divide them without exception into two neat groups we do not call them accidents any more, we call them rules. And if a rule, by definition, has a meaning, perhaps it is worth remembering that the ribotype theory is the only one which puts a meaning into that most universal of Nature's biological dichotomies.

This is incidentally the story of how this paper began. If it is speculation, so be it. But to me it looks very much like the shape of things to come.

"We all agree that your theory
is mad. The problem
which divides us is this:
is it sufficiently crazy
to be right?"

Niels Bohr

4. Discussion

During a series of informal meetings, a few friends raised a number of questions about the previous chapters which made it obvious that various points needed further clarification. I preferred, however, not to alter the original manuscript, but to add a new chapter with a list of those questions, because they seemed to me to be typical of the doubts that the reader himself might have. Some of the answers contain a certain amount of repetition, but I have left it in, in order to preserve the spirit of the original discussions.

(1) According to your theory the first cells had a sort of nucleolus and were the direct ancestors of today's eukaryotes. It seems to me that this idea will not be accepted easily. It will be very difficult to remove the concept that simpler cells originated first and the prokaryotic ribosome precursors, to give just one example, are much simpler that those of the eukaryotes.

The first computers were bulky, expensive and inefficient, and yet they came before the present microchips that you can hold on a fingernail. Admittedly, this is not an accurate analogy, because Nature worked at the molecular level since the beginning and had no need to miniaturize, but even so the example is useful. It allows us to think about the evolution of

molecular automata along similar lines, and to realize that the prokaryotes might well have been the result of a streamlining process. Another example, and a much more drastic one, is represented by the viruses which again show that what is smaller or simpler did not necessarily originate first.

But let us examine the basic reasons behind the hypothesis of the microkaryotes. I believe that a three-dimensional scaffolding was necessary to build what was going to become the precursor of a primitive cell. You cannot simply jump from molecules to cells without a suitable intermediate which provides a physical substrate for the developing cell organization and which is big enough to house within itself a complex micro-environment.

From this point of view the ability to form supramolecular aggregates becomes a precondition for the origin of the cell, and not a property that was only developed later as a result of a further evolutionary step towards a higher level of complexity. If you accept this reasoning, you realize immediately that the prokaryotic ribonucleoproteins are no good because they do not form supramolecular aggregates which reach the order of the micron.

We could attribute *ad hoc* properties to their primordial predecessors, or we could look at those ribonucleoproteins which do form the right sort of aggregates and these are precisely the ribosoids to today's eukaryotes.

At this point the argument is introduced that within the cell the molecular weight of the ribosomes can only evolve downwards and the conclusion is almost inevitable: the first cells had to have 80S-like or 90S-like ribonucleoproteins.

As for the direct ancestry, I invite you to look at the scheme of Fig. 1 which shows that the microkaryotes were the common ancestors of both prokaryotes and eukaryotes.

(2) I have found a paper which reports the molecular weights for the ribosomes of pea, sea urchin, chick and mouse as respectively 3·9, 4·1, 4·3 and 4·5 millions (Cammarano et al., 1972). That looks to me like an upward evolution of the ribosome molecular weights.

Perhaps I have not been clear enough on this point. My thesis is that if a cell increases the molecular weights of its ribosomes without gaining an increased translation accuracy, it is likely to become less competitive in the struggle for material resources. If the increase is moderate, the cell may well manage to tolerate it, but if it becomes excessive, the cell can hardly be expected to survive the competition of more efficient rivals, unless the increase is accompanied by special selective advantages.

The statement that the molecular weight of the ribosomes can only evolve downwards within a cellular framework is therefore compatible with two kinds of apparent exceptions. The first is represented by quantitative variations which account for small percentages of the total weight. The second consists of variations which are associated with qualitative advantages. The variations which you have reported may well fit into these categories because they do not exceed 15% of the total weight and because they may represent special properties that we are at present unable to appreciate. On top of that, I have said that variations of the ribotype which amount to adaptations to the cell-type are possible, and the ribotype theory therefore anticipates quite a number of minor fluctuations in the distribution of the ribosome molecular weights.

We must be careful, however, not to miss the forest because of the trees, and the secondary fluctuations should not prevent us from appreciating the basic pattern of the phenomenon. The evolution from low to high molecular weight ribosomes is the evolution from primitive ratchet-like protoribosomes whose molecular weight was presumably in the region of a few thousand daltons, to the first heavy ribosomes which had molecular weights of some millions. And the difference between 70S-like and 80S-like ribosomes is also something in the order of two million daltons per ribosome.

With the expression of upward or downward evolution of the ribosome molecular weights I was therefore referring to the massive differences which exist among the molecular weights of different classes of ribosomes, not to the minor fluctuations which can occur within each class.

If you separate the two phenomena on the basis of their different orders of magnitude you will find that there is no contradiction between the theory and the experimental data.

(3) The regulation mechanisms of protein synthesis do not form the two simple classes that you describe, and, more important, these classes do not correspond to the division of the cells in prokaryotes and eukaryotes. There are prokaryotes, for example, which have stable messengers.

My point is that 70S or 80S ribotypes were not just one of the various characteristics that prokaryotes and eukaryotes acquired after the divergence of their phylogenetic ancestors, but were the very precondition for that divergence.

Once a cell had a 70S-like ribotype it was trapped within the prokaryotic organization, which essentially means the impossibility to segregate the genome within a subcellular compartment. Does this mean that the prokaryotes must necessarily have only one regulation mechanism for protein

synthesis? No, and my theory does not say so. It simply states that a regulation based on short-lived messengers was a natural evolutionary outcome for the prokaryotes but not for the eukaryotes.

Your objection would be valid if I had stated that there is a strict one-to-one relationship between ribotype and cell-type like the one which exists between genes and proteins, but this is not the case. I have said that the ribotype provides only constraints and means for the cell-type. A 70S ribotype determines the prokaryotic organization as a class category but within this class you can have an enormous variety of different prokaryotic cells and you can equally well have a variety of different regulation mechanisms.

(4) One of your arguments is that prokaryotic and eukaryotic ribosomes are equally accurate in protein synthesis despite the fact that their molecular weights differ by one or two million daltons. To me that indicates that those differences were the result of evolutionary accidents. Why should Nature prefer 80S to 70S ribosomes in one case, and do the opposite in another case if they perform equally well during translation?

That is precisely the point. Nature did not select 70S or 80S ribosomes. Nature instead selected a 70S or an 80S type of ribosome biogenesis because it is that that makes the difference.

This point is not usually appreciated because it is thought that the only function of ribosome biogenesis is to produce mature ribosomes, and people have only looked at differences in the final products of biogenesis when they wanted to find a clue to Nature's choice. Having found that these final products perform equally well they have concluded that their differences are purely accidental. I maintain, instead, that they have a meaning but if you want to find it you have to look at biogenesis, where the differences are really significant. One type of biogenesis allows the segregation of the genome within a separate subcellular compartment, the other does not.

(5) The concept of quasi-replication has a distinct flavour of science fiction to me. How do you propose to test it if its products and processes no longer exist?

Those products and processes are anywhere there are ribonucleic acids. If you want to experiment with them you have a virtually infinite choice, from isolated nucleoli to the reconstitution *in vitro* of natural and artificial ribonucleoproteins. As for the concept of quasi-replication, you have to ask yourself one simple question: is it possible that a complete self-replicating system originated spontaneously out of a solution of macromolecules? If the answer is no, what was there before? What system could produce descendants and evolve towards higher degrees of complexity

without being a self-replicating cell? How do you define a form of replication which is not yet self-replication?

You may turn my answer down, but the problem does not go away and something very similar to what I call quasi-replication may well become a conceptual necessity.

(6) At the basis of quasi-replication there are properties like polymorphism and self-assembly, but the ribonucleic acids are not the only substances which have them. Does this not suggest that there are other candidates for precellular evolution?

You are forgetting that only ribosoids can perform protein synthesis. The crucial point is the transfer of information from the one-dimensional world of linear sequences to the three-dimensional world of physical structures and for what we know only ribosoids can bridge those two worlds. It has always surprised me that this point has consistently been regarded only as a technicality when instead it is at the very heart of the problem of life. If it had not, the ribotype theory would have been proposed a long time ago.

(7) When you talk about the origin of life, the evolution of the cells and the nature of the cell, you are describing three different theories. Are you not mixing together subjects that, for the sake of clarity, should be kept separate given our present state of knowledge? And are you not invading areas, for example cellular evolution, that are not really your field?

First of all, the three theories are described in three different chapters and are not mixed together simultaneously. If there is a common theme which runs through them that is perhaps because Nature doesn't work in separate compartments as we do in our laboratories. Secondly, I have not stolen other people's problems. I spoke on cellular evolution because the specialists there have left the ribosomes out of their schemes, and after Carl Woese that is no longer possible. As for the existing ideas in the field, I have actually shown that the ribotype theory is compatible with quite a number of them. For example it goes along well with Stanier's model and with the symbiosis hypothesis that chloroplasts and mitochondria were once free-living prokaryotes, even if it puts them in a new framework. Thirdly, and most important, the three theories are not isolated schemes; they are linked together like the rings of a necklet and you can't take one of them out without disfiguring the whole thing. For example, if I say that the ribotype is the necessary intermediary between genes and proteins, you reply that this has been known for ages and a new definition doesn't make a new concept. I have therefore to take you back in time and show what was the role of the ribotypes during evolution and during precellular

history. A cell is what it is now because of the way in which it originated and evolved, and you would have been entitled to criticize the theory if it wasn't comprehensive enough.

(8) Your attack on Oparin is not fair. He is the father of chemical evolution and has contributed more than anybody else to the problem of the origin of life. Without him the classic experiment of Miller, to give just one example, would be unthinkable.

If I were to write about the history of heredity I would have to mention that Darwin proposed the theory of Pangenesis, a modern version of a Hippocratic idea which today is totally discredited. Does this mean that I would be unfair to Darwin? Oparin has, without doubt, other scientific merits, but it is not his personality that I am concerned with, nor chemical evolution. He is the acknowledged father of what I call the phenotype theory and it is only that specific theory which is relevant to the theme of my paper.

You may have resented the fact that I linked him with Lysenko, but the fact remains that both men shared a fundamentally Lamarckian attitude towards biology, and Lamarckism is a view that we have to consider when we talk about the origin of life. I have recently received a letter from Jean Brachet who says, on this point (quoted with permission): "About Oparin, incidentally, you are right: I met him in Moscow in 1949 and he had very great sympathies for Lysenko".

(9) You must admit that Oparin, Bernal, Fox and others have presented their case with a wealth of scientific data. They have not imagined their coacervates. They have produced them in the laboratory. I have noticed, however, that in your manuscript there is only a meagre collection of experimental data.

There is no experiment that belongs to one theory alone. Any theory on any given subject must be potentially capable of explaining all the facts that are at our disposal, but this does not mean that each time we have to go through the whole list of past and present experiments to prove it.

I have mentioned just a few experimental facts simply because they were all that I needed to illustrate my point. Let me remind you that Einstein built the special theory of relativity on one simple, solid fact: the constancy of the speed of light.

(10) DNA is the basic molecule of life, and you are now saying that it has only a secondary importance. This is ludicrous.

I wish you would make the effort to see the situation in its complex reality. Does the ribotype theory state that DNA has only a secondary

importance? The answer is not a straightforward yes, as you say. The answer is yes and no, and there is no contradiction here because there are two distinct faces to the problem.

First of all the theory states that the cell is a trinity of genotype, ribotype and phenotype. Any one member of the trinity is conceptually as important as the other two, otherwise we would not have a trinity at all. Your objection therefore collapses: I do not attribute a secondary importance to the genotype as far as the nature of the cell is concerned. What I do propose is a secondary role for DNA in the history of the precellular systems. If self-replication was preceded by quasi-replication and if the ribosoids were the basic elements of the quasi-replicating systems, then it is obvious that they did have conceptual priority.

As for the relationship between ribotype and genotype during evolution, there is also a complementarity of roles and not a conflict. The ribotype channels the cell within a well defined evolutionary path. Within each path, genotype and phenotype can interact in an endless number of ways and produce an enormous variety of different cells. Any member of the trinity has its own sphere of influence and the cell is their integrated unity.

(11) On one side you attack sociobiology, on the other you accept the concept of the selfish gene which comes from sociobiology. Is not there a contradiction in that? Let me also add that the concept of the selfish gene is not universally accepted, as you seem to imply. The articles by Doolittle and Sapienza and by Orgel and Crick have stirred up quite a controversy.

I use the concept of the selfish gene because it shows that some DNA behave as molecular parasites, and I find that that is a very good analogy to illustrate how DNA originated and spread through pre-existing systems based on RNA. Since I am talking of an analogy only, it is not likely that what happens to the selfish gene has an automatic repercussion on my thesis. Nor do I see any contradiction. If I propose that DNA is a molecular parasite and sociobiology concludes that some DNA do indeed behave as parasites, that is all the better from my point of view.

(12) Sociobiology has much more to offer than you have reported. You present a very small part of it and attack the whole theory as if it consisted only of the few ideas that you mention.

You are right. The basic theme of sociobiology is the genetic basis of behaviour, but I could not mention that because it has nothing to do with the subject of the paper. Perhaps I should have made it clear that the object of my criticism is not sociobiology *per se* but the generalization that some sociobiologists have made on the origin of life and on the nature of the cell.

You must admit that the definition of the cell as a throwaway survival machine built by the genes is a pretty strong generalization and implies a very precise concept: it implies that the cell metabolism is oriented exclusively towards gene replication. To me that is a bit like saying that the purpose of all the tissues of the body is to allow the functioning of the central nervous system. Your objection, however, is right: I have discussed only a by-product of sociobiology, not its central issue.

(13) Woese is the author that you quote most, but his views are not universally accepted. The ratchet model and the division of the prokaryotes into archaebacteria and eubacteria, for example, have not received a general consensus. What happens to your theory if the concepts that you borrow from Woese turn out to be wrong?

To my knowledge, Woese has done more than anyone else to emphasize the role of ribosomes during evolution. Let me quote from p. 371 of the 1980 book on ribosomes: "The ultimate goal of biology is to explain how living systems arose on this planet; the evolution of translation holds the key to the problem". This statement was intended as a comment on the progenote theory, but it can also be used to represent, in a nutshell, the philosophy of the ribotype theory and this explains why there is an ultimate convergence of our two different approaches.

I borrow two main concepts from Woese: the first is that low molecular weight ribosomes had to originate at the precellular level; the second is that the evolution from low to high molecular weight ribosomes had to take place before the appearance of the cell biological specificity. These concepts are basic, and if they collapse so does my theory, or at least it does in its present form. All the other ideas that Woese has put forward, on the other hand, can be modified without doing any harm to the ribotype theory.

(14) It seems strange to me that you have said little or nothing about the ribosomal proteins, particularly because you are working with Wittmann who has contributed so much to this field. Do you really agree with Woese that the ribosomal proteins have only a secondary importance?

I have said little about the ribosomal proteins because the function of most of them is still unknown. In the past, people have jumped to conclusions on this subject and there have been mistakes made which I have no wish to repeat. The only sensible thing to do in this field is precisely the solid, systematic experimental work that Wittmann and his collaborators are doing. Until this work is completed, we will not be able to go into the details of the relationship between ribotype and cell-type, and it is for this

reason that my theory describes only the general outlines of such a relationship.

As for Woese's opinion on the ribosomal proteins you have to see it as a reaction which was well justified, because at that time the ribosomal RNA was regarded only as an inert scaffolding, and that idea had to be revised.

(15) In "Chance and Necessity" Monod (1971) formulated the problem of the origin of life in terms of molecular biology, and has provided a framework which I believe most scientists accept. It is not clear to me if your theory agrees with Monod's formulation or not.

With all respect, I believe that the scheme put forward by Monod is a version of what I call the genotype theory. But let us examine what he actually said. He divided precellular evolution into three phases.

(a) The first phase is the formation of nucleotides and aminoacids, the so-called prebiotic phase of chemical evolution, and here there is no disagreement. We both take chemical evolution for granted.

(b) The second phase is the formation of macromolecules capable of replication and here Monod comes very near the genotype theory. He never actually mentions naked genes but from the rest of the book it seems that by replication he means self-replication and by replicating molecules he actually intends DNA. In this case, my disagreement would be complete. At the precellular level I put the emphasis on quasi-replication, and I am not referring to molecules but to supramolecular aggregates like ribosoids and nucleosoids.

(c) The third phase of the Monod scheme consists of the processes by which the primitive replicating molecules built around themselves a teleonomic apparatus which eventually led to the first cells. Here again Monod avoids the explicit wording of the genotype theory but the spirit is very much the same. He stopped just short of saying that genes built the cell around themselves because he knew well enough that this would spell trouble. He used terms, therefore, which are flexible enough to be able to represent different things, if necessary.

In the ribotype theory the third phase of Monod corresponds to the evolution of the heterosoids, which bridge the gap between quasi-replicating nucleosoids and the first cells.

I do not know if you are able to find in his scheme parallels or anticipations of the ribotype theory, but I do not. To my knowledge Monod remained trapped in the concept of the cell as a genotype–phenotype duality, with the ribonucleoproteins in the secondary role of necessary intermediaries.

If this is true, he could not have anticipated the ribotype approach to the problem of the origins. Remember that the ribotype theory is first a theory on the cell and then a theory on the origin of the cell, even if the paper describes it the other way round.

(16) Your manuscript reports very little of the contribution which physicists made to the general principles affecting the evolution of life. For example, Walter Elsasser has written several books and papers on this.

I had to decide either for a review of for a single purpose essay, and I have chosen the latter because I believed it was a more straightforward and clear approach. The comparison with other models, the integration of various other ideas in a more comprehensive synthesis, the detailed discussion of contrasting or compatible views, are all steps which are not only interesting but essential for a profitable development of the theory. I hope I will be able to work on them in the future, preferably in collaboration.

(17) Your ideas seem to have a lot in common with those of the Gottingen–Vienna school. "Quasi-replication" would seem to describe the process by which the "quasi-species" of Eigen and Schuster replicate. The "hypercycle" consisting of nucleic acids and proteins would seem to be a version of your metaphase "ribosoids". What are the real differences between your approach and theirs?

Your statement that the two theories "seems to have a lot in common" is rather surprising, because there are only superficial similarities between them while the differences are pretty substantial.

First of all, the theory of Eigen and Schuster is based on transfer-RNA while mine is centred on ribosomes and ribosome precursors. Secondly, the quasi-species of Eigen and Schuster have analogies with the products of the statistical proteins of Woese. They are both the result of processes of "self-replication with errors", not of "quasi-replication" which is quite a different concept. Thirdly, "the component of the hypercycle had to be surrounded by a membrane" and this implies that, when the ribosomal structures developed, the evolution from low to high molecular weight ribosomes had to take place within some sort of primitive cell, while my theory requires that it took place at the precellular level. Fourthly, and most important, Eigen and Schuster do not describe the cell as a trinity, and unless you break the genotype–phenotype duality you can't have anything which resembles the ribotype theory.

All this, however, doesn't mean that my approach is incompatible with that of Eigen and Schuster. As in the case of Elsasser, the problem of extending the ribotype theory and integrating it with other ideas is very much an open one.

(18) What is the real difference between "error-prone self-replication" and your concept of "quasi-replication"?

Self-replicating systems, error-prone or not, are orientated toward the synthesis of their own components, and inevitably give origin to chicken-and-egg paradoxes. Quasi-replicating systems, instead, synthesize a wide variety of components some of which self-assemble and produce systems that function as their predecessors did, even if their constituents are all different. The problem of making more or less perfect carbon copies of the parental systems is avoided, and chicken-and-egg paradoxes therefore do not arise.

Furthermore, quasi-replication can be used by systems of increased complexity, and we can see it working throughout all the various phases of precellular evolution until it emerges into self-replication and creates the first cells.

(19) You have mentioned earlier a letter from Jean Brachet about your manuscript. Since he is one of the founding fathers of molecular biology, it seems to me that his opinion would interest quite a number of people. Can you tell us what he thinks of the ribotype idea?

Brachet's comment is this (quoted with permission): "I have very great sympathies for your ribotype theory. The reason for this is that, several years ago, I was asked to contribute a paper in honor of Oparin and suggested that RNA and ribonucleoproteins preceded DNA in Evolution. This was more for fun than anything else and I used weak arguments such as the facts that ribonuclease is thermostable and might be a very old enzyme, and that ATP (which is not mentioned in your paper) is a ribose derivative... Do not worry if your idea is criticized: it was a hard fight for me to convince the biochemists that RNA is involved in protein synthesis and this is now trivial."

(20) You cite and are obviously familiar with Popper's work. Do you regard falsification as an essential attribute of a respectable scientific theory? If so, what predictions does the ribotype theory make which could be falsified in the future?

Some falsification tests should come from the study of ribosome biogenesis. The theory implies that the mechanisms which shift the ribonucleoproteins from nucleus to cytoplasm are intimately associated with the biogenetic processes, and a detailed comparison of 70S and 80S biogeneses should reveal if that is indeed the case. It should be possible, for example, to demonstrate that eukaryotes could not survive with 70S ribosomes because they would be unable to export them to the cytoplasm.

Other falsification tests may become possible when the function of most or all ribosomal proteins will be known and the significance of the differences which exist among various species will be clarified. This should also add much more substance and content to the relationship between ribotype and cell-type that is at the basis of the theory.

Finally, we can entertain the idea that one day the manipulation of ribosomal genes may produce ribosomes which are not just variants of the existing types but which form a class of their own. In this case the theory predicts that we would have the basis for creating *in vitro* a different type of cell, a really new form of life.

(21) You present the "full ribotype theory" in Chapter 3 in the form of a philosophical or a didactic essay rather than taking (as most scientists implicitly or subconsciously try to do) a mathematical theorem as a paradigm. Your approach clearly makes better reading, but it is difficult to follow the thread of argument; consequently it does not read very convincingly to a sceptic.

Some would put your question in even stronger terms. They would say that whatever you can't put in a computer is not really a scientific problem, it's only speculation. Perhaps a future generation will be able to cope with it, but until that is possible we should concentrate on the problems that we can handle successfully by the use of appropriate algorithms. My reply would be that biology simply doesn't work that way. We would not have the cell theory, natural selection, evolution and not even the central dogma if we had to wait for their mathematical formulation.

The fact that the ribotype theory is expressed in qualitative terms therefore is not bothering me at all. What does bother me is that I might have chosen the wrong wording, I might have not been clear enough, and I know that I have only myself to blame for it. In this case, however, I would ask you to reflect before throwing away the baby with the bath water. Sit back on your chair, close your eyes and go through the arguments in your own way. You may find a better formulation of the theory, you may discover implications and developments that I have not mentioned, or you may come up with some decisive arguments against it. I assure you that I would consider all that with the greatest interest.

(22) What is "essential" to your origin of life idea?

You can see a synthetic view of the theory in the diagrams of Figs 1 and 2, if you read them carefully. Figure 2, for example, shows that there is no direct communication between genotype and phenotype.

The essential points are:

(a) the concept of quasi-replication and of the evolution of quasi-replicating systems through stages of increased complexity, all the way up to self-replication (Chapter 1),

(b) the restricted and the general version of the ribotype hypothesis (Chapters 2 and 3),

(c) the concept that the cell is a trinity and not a duality. That its life is governed by a three-way-logic and not by a dual one. In a way, one could say that the cell is a biological three-body problem.

(23) Can you describe your theory with an analogy?

I have two little stories which may give you a hint. One is about computers, the other about villages.

(a) Suppose that one day the robots take over and exterminate not only the human race but all the other forms of life. The earth becomes a sterile planet, and the robot-computers use its minerals to feed, repair and replicate themselves. The earth however can't sustain an unlimited growth of the computer population, and this produces a competition among them which, in turn, fuels their evolution toward higher types.

Eventually the computers become so sophisticated as to acquire a conscience, and start asking themselves philosophical questions like "how did we originate?". There would be two schools of thought contending that there must have been, at the beginning, either a primitive software or a primitive hardware. But eventually, being logical, they would have to conclude that what created them must have been something different.

The point is that the computers would never be able to find the answer, because the software–hardware duality is really all what they are made of. The ancestral computers had committed the original sin of destroying their creator, the intermediate member of what was once a trinity, but their descendants are unaware of this, and are condemned therefore to go on for ever wondering about "It".

(b) The second story is about communities like villages, cities and cells. A small cell contains 5 or 10 thousand ribosomes, the population of a village, while the big ones have millions of ribosomes, like the inhabitants of big cities. Imagine now to ask yourself about the origin of these communities. The first step is fairly obvious: the big cities were once small villages which just grew bigger. The equivalent is the theory that eukaryotes derived from prokaryotes, a most sensible proposition.

But what about the first villages? Here the situation gets more complicated because we have two distinct possibilities. One view is that all the different building blocks which make up the objects of the village had the

ability to aggregate and produce rudimentary houses, rudimentary machines and rudimentary inhabitants. The natural evolution of houses, beds, chairs, typewriters, TV-sets and bicycles eventually produced inhabitants that were more and more able to use them. They developed eyes to watch TV, fingers to type, legs to ride bicycles and so on. The inhabitants adapted to the evolving objects of the village and eventually achieved a perfect integration with them.

The second view states that all this is nonsense. The real things are the books. It is they who contain the instructions for making all the other objects, and these objects, including of course the inhabitants, were built with the specific purpose of producing more books. The buildings were made from drawings of the buildings to produce within them other drawings, the telephones in order to produce telephone directories, the inhabitants in order to produce books of anatomy and so on.

In a fit of madness, somebody comes out with a third idea and says that it was the inhabitants who built villages and cities, but this is promptly rejected. Everybody can see that in a city the inhabitants are going around busily making objects and are obviously the instruments with which the city keeps itself going. On the other hand, how could the inhabitants produce buildings without drawings, streets without maps, how could they do anything without textbooks, dictionaries, memos and guidelines? The suggestion is patently absurd. Its main argument is that only the inhabitants can read the instructions of the books and build objects with them. But who is going to believe it?

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