2

Proteinoid Experiments and Evolutionary Theory

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Abstract

The theory of the origin of life that has emerged from experimental reconstruction of evolution to and beyond the first cells on Earth has been collated with aspects of standard Darwinian theory. While a unified evolutionary continuum is suggested by conceptual interdigitation of origins with evolution, the premise of randomness that is central to neo-Darwinism has been found to be incompatible with the experiments.

The proteinoid theory emphasizes in an evolutionary context: non-random reactions of monomers, self-organization of polymers, endogenous control of processes, stepwiseness, cells-first, frequent events of spontaneous generation on the primitive Earth, and discontinuous evolutionary events emerging from synthesis and assembly. Some criticisms of these views are discussed. Some implications of these foci of thought for established evolutionary theory are examined.

The relationships are further examined in a context of cosmic evolution and thermodynamic principles. Deterministic influences are seen to entwine the evolutionary sequence. Definitions and connotations of randomness and non-randomness are reviewed in the light of developing evolutionary theory.

HISTORICAL INTRODUCTION

Illumination of the theory of protobiogenesis by experimental retracement of steps leading to and beyond the first cells on Earth has reflected light on

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the Darwinian theory of evolution. A guiding principle of non-randomness has proved to be essential to understanding origins; an explicit expression of this tenet supplies a long needed perspective, at least by way of emphasis, for organismal evolution. As a result of the new protobiological theory the neo-Darwinian formulation of evolution as the natural selection of random variations should be modified (Fox, 1980c) to the *natural selection of non-random variants resulting from synthesis of proteins and assemblies thereof.*

Discussions on evolutionary theory tend to become diffusely wordy because the terms used carry many connotations that differ from one evolutionist to another. The word 'random' is an example. The 'randomists' apply the term to the evolutionary matrix, to polymerization, to mutation, and other processes. Clarification of the terminology is now needed because students originally educated in such diverse subjects as biophysics, molecular biology, population genetics, origin-of-life, and electrical engineering are now coming together in a largely unorganized effort to understand evolution.

The present chapter is an attempt to present an overview of protobiological events inferred from laboratory experiments and their interpretation in the context of evolution. Data and mechanisms at the strictly chemical or physical level are presented in the relevant papers cited. Other discussions at that level are treated also in omnibus books (Fox and Dose, 1977; Matsuno, 1983). In this chapter, an attempt is made to explain relationships mainly at the evolutionary level.

Since the interpretations of the experiments and their extrapolation to evolution depend upon how randomness is conceived, or preconceived, by physical and biological scientists, some examples follow.

Monod (1971, p.98), in *Chance and Necessity* ascribed randomness to amino acid sequence in modern proteins. This is inferred from data which is consistent with similar interpretations of data collected by others (Gamow *et al.*, 1956; Vegotsky and Fox, 1962). From his factually based observation, however, Monod evidently back-extrapolated to the view that the original protein molecules were disordered. Monod expressed this relationship of primordial disorder to modern disorder by characterizing proteins as follows:

> in its basic make-up it discloses nothing other than the pure randomness of its origin.

Monod recognized that the relationship *between* (rather than within) protein molecules is non-random. For this he invoked, as have many others, the nucleic acids as the sole directing influence for a given sequence of amino acids in each protein molecule.

In at least two of his papers describing a physical approach to understanding evolution, Eigen (1971 a,b) also assumed a random matrix by declaring that 'evolution must start from random events'. In one, Eigen (1971b) wrote,

> At the 'beginning' there must have been a molecular chaos, without any functional organization among the immense variety of chemical species. Thus, the self-organization of matter we associate with the 'origin of life' must have *started* from random events.

The randomness imputed to the matrix, or what Luria (1973, p.117) has called the 'prebiotic chaos', interfaces conceptually with protein synthesis. This is evident from Crick's writing (Crick *et al.*, 1976) in which he commented on the origin of protein synthesis as a notoriously difficult problem, and excluded from consideration the formation of what he assumed to be random polypeptides.

The concept of random synthesis is repeated elsewhere; a well known book on the origin of life (Miller and Orgel, 1974), for example, assumes 'random polymers' for the early stages of molecular evolution. These various references, and others, envisage a random matrix to and including random polypeptides, which were however rescued from chaos by a nucleic acid templating mechanism. No explanation, however, is given for the origin of the templating mechanism, which must itself have been ordered, according to molecular logic.

This dominant picture of random matrix and random polymerization has led easily into the concept of random mutation which is a central tenet of neo-Darwinism. As one example, Luria (1973, p.20) states, 'the process of genetic mutation is strictly random'. The word strictly, which accurately describes much of the thinking by biologists, leaves little room for qualification of the word *random*.

However, when we turn to population geneticists and other biologists we *sometimes* read of qualifications to the term randomness. Wright (1967) for example, stated,

> The meaning of 'random' is that the variations are, as a group, not correlated with the course subsequently taken by evolution (which is determined by selection). The variations are, of course, severely limited in kind by the accumulated results of past evolution.

Gould (1982) states:

> By 'random' in this context, evolutionists mean only that variation is not

> inherently directed toward adaptation, not that all mutational changes are equally likely.

Accordingly, random does not mean random for some, whereas for the biologist Luria, for example, and the physicists it does mean random in the pure statistical sense.

Gould and other neo-Darwinists ascribe creativity to natural selection operating from without. The alternative that natural selection might operate on variants that arise from within, i.e. at the molecular level (Morgan, 1919, 1932; Haldane, 1966; Lima-de-Faria, 1962; Whyte, 1965), is not discussed. Gould's arguments are in line with Darwin's first title *The Origin of Species by Means of Natural Selection* but do not honor Darwin's later title: *The Preservation of Favored Races in the Struggle for Life*, one which suggested that Darwin himself later understood that selection of itself is not, or may not be, a creative process.

Darwin's total theory is one in which he blended the facts of variation, reproduction, and natural selection to give his contemporaries an understanding of the profusion of living things on a natural basis. By this integration he changed the course of civilization, and in fact he can be said to have introduced along with his contemporary, Gregor Mendel, a biological civilization. But his ability to process criticism by others, and to be 'self-critical', as in the amended title of his opus places him, already in his time, beyond neo-Darwinism. Had molecular science been sufficiently advanced in Darwin's day, we might now anticipate that he would also have looked at evolution from the inside out, not just the outside in.

Darwin did not use the words *random* nor *non-random* (Barrett *et al.*, 1982). Although one may impute randomistic meanings to some of his statements, it is fairer to point out that in his time related concepts were undeveloped. Thus, he stated (Darwin, undated, p.372) 'A grand and almost untrodden field of inquiry will be opened, on the causes and laws of variation . . .'

Darwin's thinking on random variation was probably closer to Luria's than to Gould's (Darwin, undated, p.16),

> We see indefinite variability in the endless slight peculiarities which distinguish the individuals of the same species, and which cannot be accounted for by inheritance from either parent or from some more remote ancestor.

And in a section on fossils (Darwin, undated, p.128),

> If my theory be true, numberless intermediate varieties, linking closely together all the species of the same group, must assuredly have existed.

Thus Darwin came close to conveying the meaning of the word *random* with his remarks on 'numberless' and 'endless' variations.

We accordingly have two principal definitions of random, as well as shaded definitions in between. In one definition, random means random, as now statistically defined. In the other, it means essentially undirected. A principal thesis of this chapter is that variations are directed, and that they are directed from the molecular level within an hierarchical organization. Either of these two definitions of *random* thus fails to fit a newer description of evolution in which self-ordered synthesis connotes much direction from the molecular level (for which explanation will be extended in this chapter). The new perspective is in disagreement with *either* random or 'undirected' variation.

An especially perceptive earlier criticism of randomness in evolution was that of Eden (1967), who stated,

> Any principal criticism of current thoughts on evolutionary theory is directed to the strong use of the notion of 'randomness' in selection. The process of speciation by a mechanism of random variation of properties in offspring is usually too imprecisely defined to be tested. When it is precisely defined, it is highly implausible.

It is the discovery that amino acids are self-sequencing that is responsible for our emphasis on non-random variation and for the view that evolution is self-limiting, in contrast to the widely prevalent assumption of random variation. When the inference of non-randomness was bolstered by a newer perspective of cosmic order derived from the Big Bang (Fox, 1980b), the possibilities for quantitative testability of evolutionary sequences and theoretical testing of modelled processes (Matsuno, 1982) emerged.

The phrase 'natural selection of random variants' is a compressed statement of Darwin's theory. It fits the original Darwinism approximately as well as it fits neo-Darwinism. As had to be learned after Darwin, causes are fundamentally genetic and even more fundamentally molecular.

In this respect, it is of historical interest to examine the views expressed in T. H. Morgan's (1932) writing on *The Scientific Basis of Evolution*, published after Morgan had consolidated his theory of gene linkage in the chromosome.

In that book, Morgan wrote, 'Neither the genetic factors responsible for a part of the initial variability, nor the environmental factors can bring about such an advance'. Morgan was here stating that the process of natural selection is incapable of moving variability beyond determinate limits. This view has been disputed (Mayr and Provine, 1980).

Some modern geneticists believe that 'modern genetics' has explained

variation adequately. They point to recombination, the founder effect, genetic drift, and punctuated equilibria; these have even been summarized in a legal opinion (Overton, 1981).

None of these processes was totally unfamiliar to Morgan; there is no reason to believe that they would have led him to change his evaluation, especially when the processes are rationalized as having arisen from a random matrix, i.e. by chance. We may look elsewhere for causes of variation, especially in the internal non-randomness that Berg (Haldane, 1966, p.12) and Morgan[1] favored (cf. Mayr, 1980). A fresh source of such perspective is the sharpening picture of protobiology.

Accordingly, we shall examine the model for protobiology in some detail and then analyze its relationship to the Darwinian concepts.

HOW DO WE LEARN HOW LIFE BEGAN?

The problem of how life began has long been considered to be imponderable. Much of the reason for this is that modern science is predominantly reductionistically analytical. For many modern scientists, an adequate feeling of assurance can be obtained only by analyzing what is here in hand.

We do not however have any primordial organisms to analyze. All we have are distant descendants of primordial organisms. Even if we had on hand a primordial organism, a complete analysis would be a long way from answering how the components identified by that analysis were assembled into that first organism. What could be learned we would learn by 'taking apart'; what we want to know requires 'putting together'. The latter is synthesis and assembly, or *constructionism* (Fox, 1977).

However, the starting point for constructionistic studies was analytical. The basic enabling principle is the 'unity of biochemistry' (Florkin and Mason, 1960). The labours of thousands of analytical biochemists have taught us that all life, all living units, are constructed in an amazingly ramified but highly limited variety from a remarkably small number of molecular units: nucleic acids from five main nitrogen bases; proteins from twenty amino acids; lipids from three components of which the fatty acids are fewer than ten kinds; and carbohydrates from a handful of monosaccharide types (Lehninger, 1975; Needham, 1965). The principle of the unity of biochemistry is perhaps the most pervasive evidence that evolution has occurred by descent with modification at the molecular level.

Had there not been a discernible unity underlying biochemical variety, living things would have seemed chaotic and perplexing, and in such a

situation, the prospects for selecting a protobiochemical model would have indeed been hopeless. Since organisms are so much alike chemically the possibility for experimenting with protobiochemical recipes was encouraging, especially when aided by some inferred geochemical guidelines.

The other potential approach is that of back-extrapolation from what is known of modern organisms, a large catalog of knowledge indeed. Crick (1981, p.37f) has analyzed the problem similarly, but he does not bother to consider the investigative approach of back-extrapolation, which obviously cannot identify an initial assembly mechanism (Fox, 1977). He does, however, seem to be sufficiently dismayed by the difficulties of a constructionistic approach that he prefers to devote his efforts to explaining how life arrived here from elsewhere. Perhaps this is not so much dismay as the exercise of a characteristically theoretical, rather than an experimental, approach to problems. Crick (1981, p.148) acknowledges that many scientists plus his own wife regard 'directed panspermia' as 'science fiction'.

The panspermia concept stems from Svante Arrhenius (Oparin, 1957); it received much attention in an era which could look back upon no experimental investigation other than the discredited nineteenth-century experiments of 'spontaneous generation' (Oparin, 1957). Since a modern kind of experiment on chemical spontaneous generation began in the middle of this century (Fox, 1957), numerous eminent scientists have turned their attention to the problems of the origins of life and of the genetic code (e.g. Crick, 1968, 1970; Calvin, 1969; Monod, 1971; Eigen, 1971a,b; Luria, 1973; Nicolis and Prigogine, 1977). The majority of the authors cited used the Aristotelian approach of developing a picture by purely theoretical means from data obtained from modern organisms. Their assumptions about the original matrix are neo-Darwinian. The *experimental* approach has however highlighted the inadequacy of neo-Darwinian principles and has provided a theory of a markedly different kind. The differences will be analyzed here after the comprehensive (Lehninger, 1975; Florkin, 1975) experimentally derived theory is itself described.

MOLECULAR EVOLUTION TO LIFE

The flowsheet of primitive molecules to protocells is seen in fig. 2·1. This flowsheet was established by over 200 man-years of experimental exploration.

The evidence for the existence of sets of amino acids is from returned samples from the lunar surfaces and meteorites, and from numerous simulation syntheses (Fox *et al.*, 1981).

The fact that sets of amino acids containing trifunctional types (aspartic acid) undergo copolymerization in a non-random manner is crucial to molecular evolution and to all of evolution; the evidence for such self-ordering is firmly established from experiments in numerous laboratories (Fox, 1980b). Numerous other studies have also shown that the highly specific polymers obtained have 'protoenzymic' activities (Fox, 1980a). The

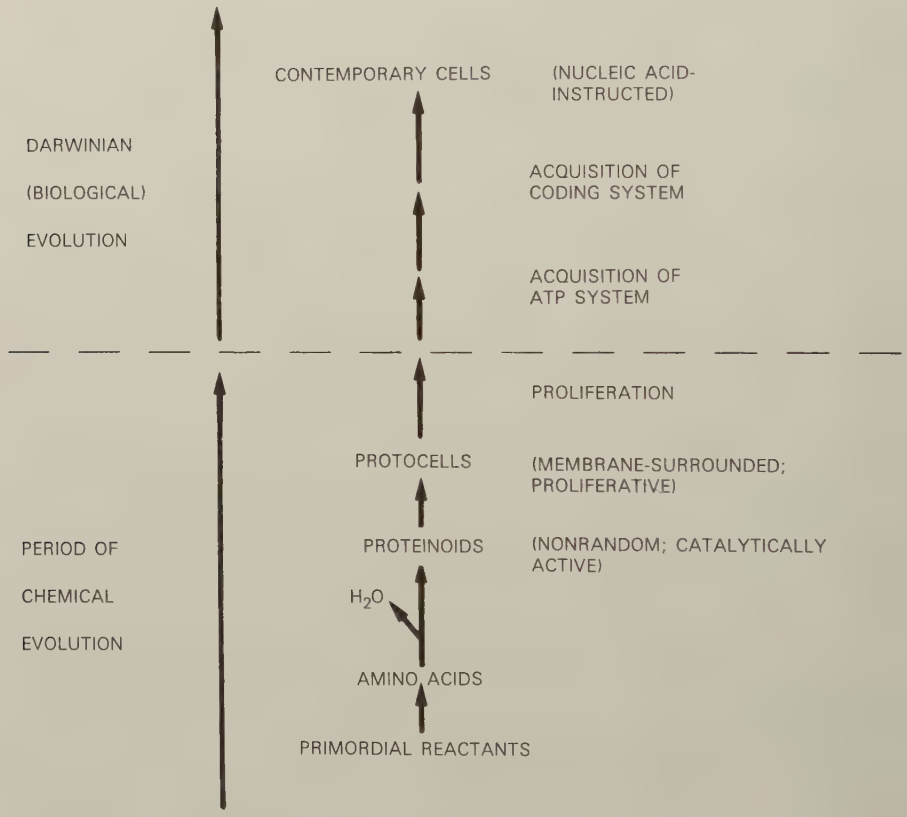

FIG. 2·1 Flowsheet of molecular and protocellular evolution (Fox, 1981c) from primordial matter.

polymers or proteinoids (thermal proteins), are catalytically active in various specific ways due to the interactions of amino acid sidechains, much as in modern proteins (with amplification of functional diversity by metal ions and other prosthetic groups).

The polymerization of amino acids is operationally simple enough, but

the self-organization of proteinoids to simulated protocells in the laboratory is simpler yet. Mere contact with free water is all that is necessary (Fox, 1976). The tendency to reproduction involving heterotrophic growth has been demonstrated by binary fission (Fox and Yuyama, 1964), budding (Fox, McCauley, and Wood, 1967), and other means (Hsu *et al.*, 1971; Brooke and Fox, 1977).

One remarkable aspect of the simulated protocells is their simultaneous content of varied activities. This perspective is a revelation; it contrasts with the assumption of Oparin (1957) that molecular order and function would have had to evolve in the protobionts he modelled. It must be remembered, however, that Oparin's coacervate droplets could not assume the role of protocells since they were produced from evolved biopolymers instead of from the equivalent of protobiopolymers.

In modern cells the barrier function of membranes depends upon phospholipid and the order of amino acids in proteins results from the functions of nucleic acids. In the modeled proteinoid protocell, however, the order of monomers is due to the self-sequencing of the amino acids (Fox, 1978), and the membrane of the microsphere is proteinoid (Fox *et al.*, 1982; Przybylski *et al.*, 1982). Accordingly, a functional proteinoid protocell required neither nucleic acid nor phospholipid. The protocell had, moreover, according to the experiments, an ability for (heterotrophic) growth (Fox *et al.*, 1967; Fox, 1973a).

In the light of this analysis, the probability that the proteinoid microsphere represents the original terrestrial protocells, and that no other entity could fill that role, is much greater than if primitive types of protein, nucleic acid, and lipid were each to have arisen separately and then to have coassembled.

Once the functions of the laboratory protocell had been catalogued sufficiently, it became clear which functions were required in order to bridge the gap from modeled protocell to modern cell. Outstanding among these requirements were the abilities to synthesize proteins and to synthesize nucleic acids. For example, a principal difference between the reproductive mode of the proteinoid microsphere and that of the modern cell is that the latter synthesizes its own proteins (Weissbach and Pestka, 1977), whereas the former obtains its proteins in a preformed state from the environment.

What has been learned recently is that proteinoids rich in basic amino acids are capable of catalyzing the synthesis of peptides and of enlarging the peptidic proteinoids themselves by conjugation with amino acids or peptides; ATP or pyrophosphate (Baltscheffsky, 1981) must be supplied as energy source. They are capable also of catalyzing the synthesis of polynucleotides (Fox *et al.*, 1974). Since the processes of synthesis of protein and of polynucleotides are catalyzed by the same basic polyamino

acid and the catalysts are effective within microspheres (and within complexes with polynucleotides) the proteinoid microspheres on the early Earth could have served as effective locales for the evolution of the genetic mechanism and code. This sequence is represented in fig. 2·2.

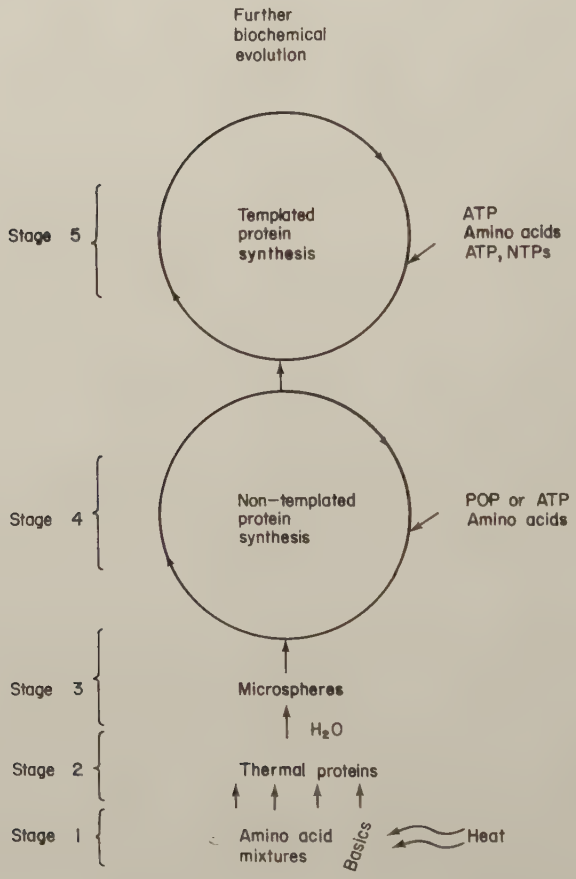

FIG. 2·2 Flowsheet of experimentally suggested evolution from reproductive protocells to modern cells. Thermal peptides were synthesized in geological stage 1. Some of the mixtures contained sufficient basic amino acid for the early part of stage 4 and stages 2 and 3. In stage 5 some of the ATP, and other nucleoside triphosphates, polymerized to polynucleotides in microspheres. In stage 4, large peptides would presumably yield offspring microspheres Fox, 1981).

LESSONS FROM PROTEINOID EXPERIMENTS

Ease and speed of formation of protocells

An outstanding feature of the experimental findings is the ease with which proteinoid microspheres possessing remarkable properties can come into existence. The expectations were quite otherwise; the view was that anything as complex as a cell could have arisen only after a prolonged and intricate evolution. An example of this premise was a detailed symposium in 1964, titled *Evolving Genes and Proteins* (Bryson and Vogel, 1965), at which it was possible to point out that genic and enzymic evolution must occur within *cells* (Fox, 1965; cf. Prosser, 1965)—entities omitted from the title. If one focuses attention on a modern, evolved cell, the need for prolonged, intricate evolution appears to be justified. That the evolution could occur from a less developed cell is the saving concept.

The expectation that the origins of cells would be rare and would require a long evolution (Crick, 1981; Eigen *et al.*, 1981) is a plausible one. Eigen *et al.* (1981), for example, state, 'Organization into cells was surely postponed as long as possible'. The arbiter for the correct point of view must however be the results of experiments, and experiments only. Although it is not always possible to determine where premises end and experimental results begin, the ease of formation of *proto*cell early in molecular evolution must stand out as a view firmly supported by experiment.

Neither scientific logic nor data permit the instantaneous origin of a fully evolved modern cell; that would have been instant creation. The modern cell accordingly evolved from the protocell by a series of *self-organizing steps* (Fox, 1960). The protocell can thus be thought of as discernibly related to the modern cell, albeit deficient in some functions, either quantitatively or qualitatively.

Number of protocells

Another aspect revealed by experiment is that protocells would have arisen in astronomical numbers. Prior to the first experiment, this is not what was expected (Fox, 1983a). However, 1 gm of proteinoid can yield 10^{10} microspheres.

Sociality of protocells

Because of large numbers in a dense population, the kind of sociality described by the psychologists Tobach and Schneirla (1968) would have

been present in the first organisms. In view of the obvious tendency of all living forms to join into societies large or small, the observation of this phenomenon in laboratory protocells, as representative of initial behavioral units, was arresting. Tobach and Schneirla (1968) stated:

> Until forms intermediate between organic and inorganic matter are better known, the fact that all cells issue directly from other cells may be taken to indicate that no existing form of life is truly solitary, and no organism is completely independent of others at all times in its history. This dependence of every individual on others is the prerequisite to social behavior.

With the development of protobiology and the opportunity to examine the behavior of laboratory protocells 'intermediate between organic and inorganic matter', this intimation of Tobach and Schneirla was borne out. Laboratory protocells display strong tendencies to associate and to communicate (Hsu *et al.*, 1971). The individual microspheres not only influence each other, they associate in ways that constitute behavior that could not be predicted from examining individuals alone (Fox, 1983a). The experiments even suggest parent–offspring relationships that have not earlier received attention—that many of the first or originally predominant parent–offspring relationships were those of foster parent-children.

Physical properties, general

The properties that have been described in previous paragraphs have been selected for their relevance to evolutionary theory. The other properties that have been characterized are numerous, and are available for perusal in monographic treatments (Fox and Dose, 1977; Fox, 1978).

It will be of value however in a chapter to be read by evolutionists again to correct some misleading statements, exemplified by those that appeared in a recent book on evolution (Stebbins, 1982). One statement claims that 'the majority of protenoid (sic) spheres show no chemical activity at all, and only a few have a weak enzymic activity'. Another implies the lack of ordered sequence in proteinoids and a third assigns a short life to microspheres.

Chemical activity is however found in all microspheres (Fox, 1980a) in which it has been sought. To assert the opposite, as Stebbins has done, is merely to indulge in negative armchair ratiocination.

The assertion that proteinoid microspheres are short-lived is also false. Aseptically prepared microspheres (bacteria from the air can feed on proteinoids) have been observed to last for six years before they were discarded (Hsu and Fox, 1976). The statement that proteinoids will

thermodynamically tend toward hydrolysis (Miller and Orgel, 1974) may be 'theoretically' justified, but this ignores the realities of kinetics and of stabilization by intramolecular interactions in proteinoids, not to mention the published experimental evidence.

The mis-statement about amino acid sequence ignores the self-ordering power of amino acids, which is a thesis of this chapter and is now seen as a cornerstone of any valid theory of evolution.

These errors are illustrative of the kinds of difficulty that non-chemists sometimes encounter in incorporating chemical or biochemical facts into the generally sought evolutionary synthesis (Mayr and Provine, 1980). On the other hand, chemists and biochemists have themselves not aided the enterprise, since nearly all of them have avoided evolutionary theory until the 1950s. Even then, many tended to look on evolution as a term to be uttered like a password that would let them enter through the dark green door of a 1930s speakeasy. Some of the shining exceptions to this last generalization were Henderson (1913) and Joseph Needham (1935); and later on Florkin and Mason (1960) and Dayhoff (1972) and associates.

Other objections have been systematically discussed elsewhere (Fox, 1983b).

Main theoretical advances

The principal advances that can be credited to the proteinoid experiments are perspectives that were attained either earlier than by any other approach or *only* because of the exploratory experiments. The main advances listed below are all conceptual, but are derived from the physical advance of the production in the laboratory of an evolvable organized cell-like structure. This structure is composed of internally ordered macromolecules (thermal copolyamino acids), which support in the structure primitive aspects of metabolism. The microstructures have double-layered boundaries, they can participate in reproduction, and they have many, if not all, of the properties found in modern cells in some primordial form. The physical advance occurred more than twenty years ago (Fox, 1981a), when its general or detailed nature was barely understood. The conceptual advances resulting therefrom are much of what will be discussed here.

The self-sequencing of amino acids

The fact that amino acids are self-sequencing is the essence of an evolutionary theory that takes us beyond, and out of, neo-Darwinism. It is also, in hindsight, what made possible the production of a laboratory protocell and the related idea of the spontaneous generation of cells on the primitive Earth 3–4 billion years earlier. This is the crucial phenomenon in

the understanding of protobiological evolution (Fox, 1978). It is the products of self-sequencing, the thermal proteinoids, that explain the origin of enzymes, the origin of cells, and the ability of such cells to make the materials, with controlled help from the environment, that could yield a continuing stepwise generation of evolving cellular organisms.

The initial experiments that explained self-sequencing, or self-ordering (Fox, 1960), were based on theoretical inferences derived from an earlier kind of experiment in which self-ordering was observed. The impetus for continuation throughout was a stream of new suggestions from experimental results.

The first experiments had objectives in protobiology. They were experiments on the synthesis of substituted peptide derivatives in aqueous solution in the presence of proteolytic enzymes (Fruton, 1982). Some of the experimental results suggested that the identity of the substituted peptide products was a function not only of the enzyme, but of the small molecules that constituted substrates (Fox *et al.*, 1953). It has been fashionable to speak of the specificity of proteolytic enzymes, a phrase that immediately tends to suggest that no other component of the reaction system contributes to the specificities in synthesis or degradation by enzymes. With the experimentally derived inference that (substituted) amino acids could, along with enzymes, contribute to the specificity of reactions, the way was cleared for testing the interreaction effects of amino acids alone, *without enzymes*, in the formation of specific acellular proteins (Fox, 1956). The self-ordering was then observed (Fox *et al.*, 1953); like the polymerization, such specific behavior did not require enzymes. Moreover, the origin of specific enzymic activity in the polymeric products could be visualized and tested from this enzyme-free matrix. Activities, albeit weak ones by standards of evolved modern enzymes, were found in abundance (Rohlfing and Fox, 1969; Oshima, 1971; Dose, 1983; Fox *et al.*, 1974).

Most of the first indications that the polyamino acids are ordered emerged in several laboratories from fractionation of proteinoids (Fox, 1981b). Subsequently most of the evidence that self-ordering is a general process including self-sequencing of the amino acids (Nakashima *et al.*, 1977; Hartmann *et al.*, 1981; Melius, 1977) was reported (Table 2·1).

While the sequences reported as dominant are two tripeptides, peptides of 10 000 molecular weight or more are known to arise from thermal condensation of amino acids (Fox and Harada, 1960; Melius and Sheng, 1975). The explanation for the recovery of molecules as small as tripeptides seems to be that the polyamino acid chains are punctuated by molecules like flavins and pteridines (Heinz and Reid, 1981); it is to be expected that the linkages between amino acid residues and the heterocycles would split most easily.

TABLE 2·1 Tyrosine-containing tripeptides found versus those expected on the basis of the random hypothesis

Expected from *random* polymerization		*Found* from *non-random* polymerization
αUαUY	YαUU	
αUγUY	YγUU	
γUαUY	YαUG	
γUγUY	UYγUG	
αUGY	YαUY	
γUGY	YγUY	
αUYU	PαUY	
γUYU	PγUY	
αUYG	PGY	PGY
γUYG	PYU	
αUYY	PYG	PYG
γUYY	PYY	
GαUY	YGU	
GγUY	YGG	
GGY	YGY	
GYU	YYU	
GYG	YYG	
GYY	YYY	

The dominant fraction obtained from the thermal copolymerization of glutamic acid, glycine, and tryosine proved to be an equimolar complex of pyroglutamylglycyltyrosine and pyroglutamyltyrosylglycine (Nakashima *et al.*, 1977; Hartmann *et al.*, 1981). U = glutamic acid residue, Y = tyrosine residue, G = glycine residue, P = N-pyroglutamyl.

The results of the sequence study reported in Table 2.1 are not the only ones derived from analysis of terminal residues. Striking examples of non-randomness are found in the studies of Melius and Sheng (1975), and in other analyses (Fox, 1980c).

Moreover, numerous other kinds of study have verified the basic processes of self-ordering (Fox, 1980b; Fox, 1981b).

The fact that the ordering could occur without nucleic acids to guide it resolved a principal problem of origins, the so-called chicken–egg question (Lehninger, 1975; Eigen, 1971a; Florkin, 1975).

Early formation of a cell

Viewed as a problem in pure Aristotelian logic, the emergence of the cell should have followed that of the cell's components. This appears to be logical on the basis that the totally assembled cell is a final packaging of all its component molecules. Luria (1973, p.115) has, for example, explained the difficulty of direct genesis of a modern cell in that cells as we know them are enormously complicated objects.

Our experiments, however, suggested another sequence. Thermal proteins

alone aggregate on contact with water with consummate ease. Upon experimental extension of this unexpected revelation, a new picture formed of an early cell that could then evolve to a modern cell. During that evolution, and probably early in that evolution, the evolving cell learned to make both its own protein and its own nucleic acids and other components (Fox, 1981c). The evolving cell then passed through a stage of non-templated protein synthesis, into templated protein synthesis, as explained in detail elsewhere (Fox, 1981c).

A principal rethinking required by the recognition that the cell emerged early is that required for evolutionary processes in general—that the primordial processes must be understood and mimicked in a manner that is partly detached from modern circumstances (Fox, 1981a). The protocell, a first cell, could have emerged without having had all of the properties of the modern cell, or without having any or all of those functions in as full measure or as specialized as they are found to be in the modern cell.

Also, one cannot extract any protein from modern organisms and cause it to form cell-like structures in the simple, efficient, largely encompassing way that thermal proteins perform this act. As proteins became more specialized in evolution, many evidently lost this propensity.

Numerous associated properties in the protocell

The lengthy roster of properties and functions that the proteinoid microspheres are found to have, include manifold protoenzymic, protophysiological, and protobehavioral types (Table 2·2). The evidence in the literature is keyed in reviews (Fox and Dose, 1977; Fox, 1978; Fox *et al.*, 1978; Fox and Nakashima, 1980).

The accumulated knowledge has had to contend with assumptions constituting a belief that the first cells were inert, and that the typical properties of cells as we know them were developed during the evolutionary steps. In other words, not only was the cell assumed to have arisen late in molecular evolution, the functions of the evolved cell were assumed to have been acquired entirely during evolution.

This latter view was due largely to Oparin's studies. It is true that the role of Oparin's coacervate droplets as models for protocells, his 'protobionts', has often been regarded as equivalent to that of proteinoid microspheres; the two models are treated side-by-side in textbooks (Lehninger, 1975; Korn and Korn, 1971; Scott, Foresman and Co., 1980). Some authors however recognize that some of the differences between standard coacervate droplets and proteinoid microspheres are crucial.

Coacervate droplets have typically been made from materials like gum arabic and gelatin, oppositely charged colloids (Oparin, 1957, p.301) obtained by extraction from highly evolved organisms. Their use fails to

TABLE 2·2 Associated properties in the laboratory protocell

In proteinoids	*In microspheres*
Enzymic	
Esterolytic	Esterolytic
Phosphatatic	Phosphatatic
Decarboxylatic	Decarboxylatic
Aminatic	
Deaminatic	
Peroxidatic	Peroxidatic
Peptide-synthetic	Peptide-synethetic
Internucleotide-synthetic	Internucleotide-synthetic
Photochemical	
Decarboxylatic	
Hormonal	
MSH	
Behavioural	
Electrophoretic	Electrotactic
Enzymic	Enzymic, metabolic
Aggregative	Aggregative, thigmotactic
	Motile
	Osmotic
	Selectively diffusive
	Fissive
	Reproductive
	Conjugative
	Communicative
	Protective
	Excitable

[a] A review of the protobehavioral properties is found in Fox and Nakashima (1980).

illuminate how cells came into existence when there were in existence neither cells nor cellular polymers. Moreover, to perform some highly interesting experiments, Oparin (1971) incorporated enzymes from modern cells.

Against this background it is easier to understand why Oparin (1957, p.290) believed,

> All that we can expect . . . is (to) . . . explain the formation of organic polymers in the shape of polypeptides and polynucleotides, assemblages having, as yet, no orderly arrangement of amino acid and nucleotide residues adapted to the performance of particular functions.
>
> These polymers were, nevertheless, able to form multimolecular systems. . . . It is only by the prolonged evolution of these . . . that there

developed . . .: metabolism, proteins, nucleic acids and other substances with complicated and 'purposeful' structures. . . .

This view is the logical inference from data on coacervate droplets.

The proteinoid microspheres, which are historically more recent, represent the emergence of protocells from precursors formed in the geological realm, proteinoids. These proteinoids and the aggregation products thereof (Fox, 1980a), are crowded with functions.

Salient functions within a protocell

The association of functions in the proteinoid laboratory protocell is best understood by focusing on those functions which are considered in the modern view to be essential to the living system. These are (a) ordered macromolecules, as in the coded genetic system, (b) metabolism from the actions of specific enzymes, and (c) membranes of cells, all in association. All of these are present simultaneously in proteinoid microspheres with no other substances included, which is not true in evolved specialized proteins. This strengthens the view that proteinoid microspheres could have evolved to modern cells with the same functions provided by three types of modern substance: protein, nucleic acids, and lipids.

TABLE 2·3 Evidence for the membrane nature of the boundary of proteinoid microspheres

Electron micrographs (Fox and Dose, 1977)
Selective permeability (Fox *et al.*, 1969)
Osmotic properties (Fox *et al.*, 1969)
Black films (Dr. Gilbert Baumann in Fox *et al.*, 1978)
Polarization in microsphere boundary (Ishima *et al.*, 1981)
Electrical discharge from microsphere boundary (Przybylski *et al.*, 1982)

That the protein functions can be provided by proteinoids was the easiest to grasp and the first to be established (Rohlfing and Fox, 1969). That the proteinoids could be ordered macromolecules having no DNA nor RNA in their evolutionary history required the solution that the first information came from reactions of simpler substances, the amino acids. The surprising aspect of this was the high degree of precision in self-ordering. That the proteinoid microspheres had at least moderately efficient

membranes without the benefit of modern phospholipid was established by several lines of evidence, not the least of which was the strong electrical behavior observed (Table 2·3).

TESTS OF VALIDITY OF THE THEORY

Science proceeds from initial periods of direct observation to admixtures of observations and inferential interpretation. The substance of protobiology represents a discipline that has appeared within science as a whole when the latter was already well stocked with data from geology and biochemistry. Atop this base were introduced corrective inferences derived from building models of the origin of life. The corrections ensued because the knowledge gained by analysis of the objects of geology, biochemistry, and physiology could not explain how these objects came into existence by what was, in a sense, a process that was the inverse of analysis, i.e. synthesis and assembly.

Some tests for validity have however been available.

Microfossils

One may ask whether any objects resembling the proteinoid microsphere (interpreted as laboratory protocells = units of protolife) are found in ancient strata. This question is thus phrased in a proper historical sense, since the proteinoid microspheres were reported in 1959 (Fox *et al.*, 1959) whereas the spheroidal microfossils were first reported in 1962 (Barghoorn and Tyler, 1963). The possibility that the microfossils are lithified relics of spontaneously originating proteinoid microspheres was suggested at the same conference in which the microfossils were first reported (Fox and Yuyama, 1963a).

To at least one physiologist (Keosian, 1974), this explanation (Fig. 2·3) appeared likely. Following the application of a new method of artificial fossilization (Leo and Barghoorn, 1976) to both algae and proteinoid microspheres, a group of micropaleontologists (Francis *et al.*, 1978) reported that artificially fossilized proteinoid microspheres and artificially fossilized algae resembled each other. The two sets of objects could not be distinguished. Other micropaleontologists (Cloud and Morrison, 1979; Pflug and Jaeschke-Boyer, 1979) have recorded similar judgments.

In addition to the spheroids, microfossils caught in the act of binary fission (Knoll and Barghoorn, 1977) were imitated, in advance of their finding, by proteinoid microspheres. The proteinoid microspheres can be studied dynamically, which is of course not true for fossils. Such study has

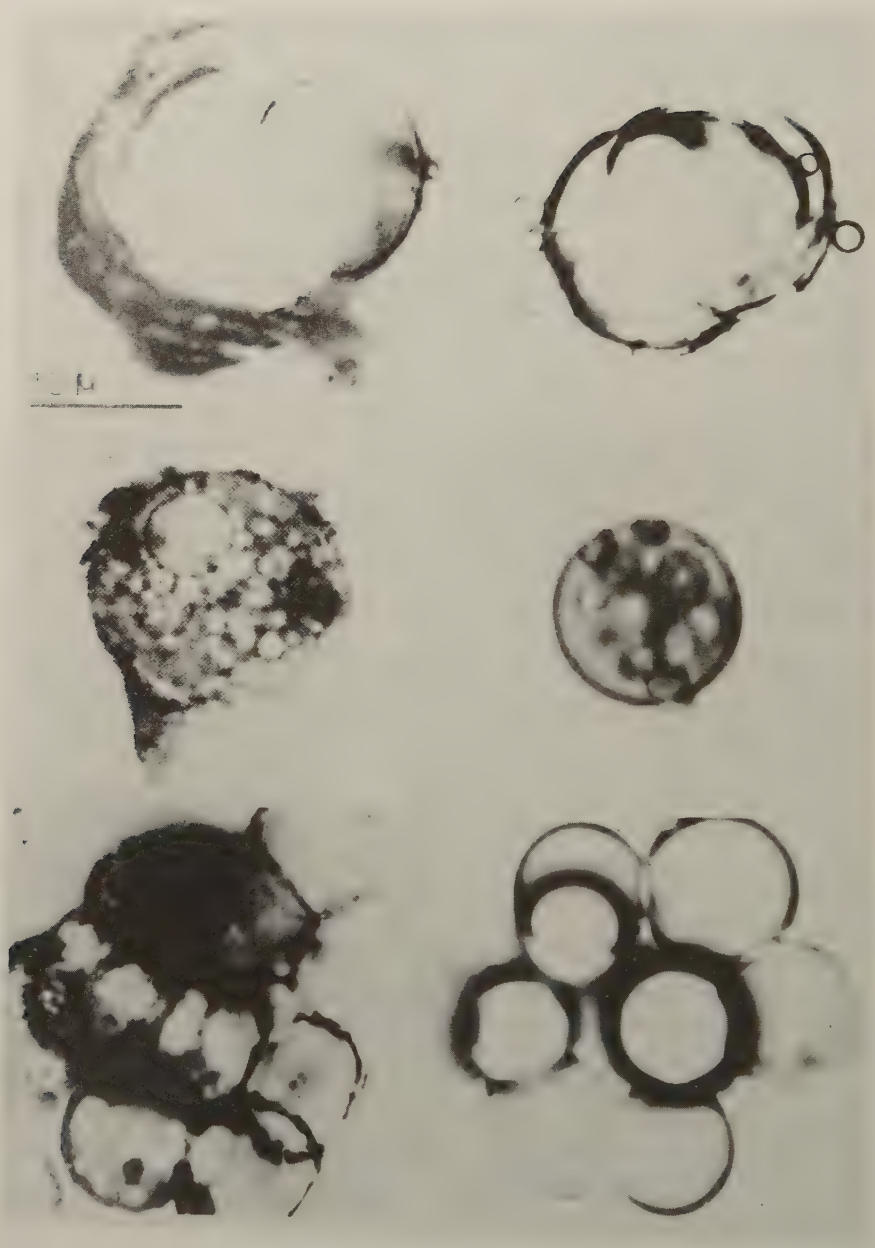

FIG. 2·3 Comparison of ancient microfossils (on the left) with proteinoid microspheres (on the right).

revealed that some microspheres that appear to be dividing are actually two microspheres undergoing coalescence. Although that was not offered as an alternative by Knoll and Barghoorn, it cannot be ruled out by morphological examination of the twinned microfossils.

De novo organisms from hydrothermal vents

The micro-organisms that appear in events from hydrothermal vents have been proposed as being closely related to proteinoid microspheres (Corliss *et al.*, 1981). Part of the reason for this proposal is that such events have a thermal beginning followed by processes that occur in less time than a diurnal cycle, and which can occur in the dark. These conditions are met by the proteinoid microspheres (Fox and Dose, 1977). The field occurrences have been recorded or suggested for the Galapagos Rift, the East Pacific Rise, Spirit Lake near Mt. St. Helens (Corliss *et al.*, 1981), and earlier in a similar geological context for Yellowstone Hot Springs (Copeland, 1936). The function of a submarine volcano or hydrothermal vent for protobiogenic occurrences had been predicted earlier from observations in the laboratory (Fox, 1957; Mueller, 1972).

Prions

The prions are pathogenic organisms responsible for diseases such as scrapie (Griffith, 1967; Prusiner, 1982). Although purified only with great difficulty, the prions have many properties in common with proteinoid microspheres as indicated in Table 2·4. The scrapie organisms, however, are much smaller in size than the proteinoid microspheres.

TABLE 2·4 Properties common to prions and to proteinoids and microspheres

Stickiness [aggregation and thigmotaxis in microspheres (Kimberlin, 1982; Fox and Nakashima, 1980)]
Molecular weights below 50000 (Prusiner, 1982; Fox and Dose, 1977)
Ability to reproduce (Prusiner, 1982; Fox, 1974)
Physical stability (Kimberlin, 1982; Fox and Dose, 1977)
Nonantigenicity (Prusiner, 1982; Fox and Dose, 1977)

Although neither Prusiner (1982), nor a commentator on his studies (Kimberlin, 1982), has published any statement about such a relationship, the possibility of a close relationship between prions and proteinoids and microspheres has suggested itself. This similarity in unique properties is seen in Table 2·4. Such similarity does not constitute evidence in the way that a comparison of artificially fossilized algae and artificially fossilized microspheres does. However, it should be considered with that evidence and with the suggestions made for products from hydrothermal vents. What also should be considered is the considerable disparity in size between scrapie units and proteinoid microspheres.

The significance of heritable or endogenously similar protein production to prion replication has been explained by Melius (1982) as due to self-ordering of amino acids, and by Root-Bernstein (1983) as due to amino acid complementarity. Each mechanism operates without nucleic acids. Melius reviews other known mechanisms for non-ribosomal synthesis of peptides in organisms.

COMPATIBILITY OF PROTOBIOLOGY WITH BIOLOGY

One fundamental general test for validity that must be passed is whether the principles and phenomena of protobiology bridge to the principles and phenomena of biology.

A number of such relationships are documented here. In each case, the protobiological tenet was proposed, or largely emphasized, before the counterpart biological tenet received widespread attention.

Microfossils

The resemblances between microfossils and lithified proteinoid protocells has been pointed out above. The proteinoid microsphere as a protocellular bridge between protobiology and biology is thus supported by circumstantial evidence.

The concept of proteins-first

The two great classes of informational biomacromolecule are the nucleic acids and the proteins. The question that has long evaded resolution is which of these is primary in the biochemical economy of the cell and which was primary in evolution. The general consensus of understanding in biochemistry is recapitulationist, i.e. is such that biochemical sequences are

expected to have arisen from a similar sequence of earlier evolutionary events.

The idea that protein and nucleic acids are both primary is not new. Commoner has been a leading proponent of joint primacy. In a paper in which he first pointed out that inheritance is uniquely associated with life (life is much more than inheritance!), Commoner (1968) said, 'the biology of inheritance is embodied not only in nucleic acids but in proteins as well.'

The narrower point of view, i.e. the tenet that nucleic acids or, more specifically DNA, are primary has been prevalent. This perspective was early fostered by Muller (1955), and it continues into recent times as can be seen in Crick's (1981) book *Life Itself*. Leaders in theoretical construction: Monod (1971), Eigen (Eigen and Schuster, 1979), Luria (1973) have all treated the subject so as to help strengthen the DNA-first concept. This thesis is aided also by companion assumptions of randomness, DNA self-replication and contexts that treat evolution as only selection, and life as only inheritance (cf. Fox, 1981c), none of which is justified by supporting treatments.

Controversy over these opposing views has erupted from time to time and Commoner's interpretations were 'shouted down', according to Hubbard (1982). One of the few authors to analyze each of the points of view with considerable impartiality is Lehninger (1975).

The proteinoid results have led directly to a view of proteins-first from the outset of evolution (Fox, 1960). But that experimentally derived mechanism is not a simple direct-line relationship. Rather, it is at least a two-stage development in which first arose *thermal* proteins (proteinoids) that gave rise to *protocells* that in turn had the power to make true cellular proteins, and also had the power to make polynucleotides. It is this *stepwise* sequence of events, modelled in large part by experiments, that made the general idea of proteins-first a viable one.

More recently, the work of Kornberg and others on DNA polymerase has highlighted the primacy of the enzyme proteins in modern systems. Kornberg (1980) has stated the position clearly with the words,

> It was suggested in 1953 that A-, T-, C-, and G-containing precursors might orient themselves as base pairs with a DNA template and then be 'zippered' together without any enzyme action. However, to the biochemist it is implicit that all biosynthetic and degradative events are catalyzed by enzymes, making possible refinements of control and specificity, and rapid rates of reaction.

The key point is that the protein → nucleic acid sequence established by proteinoid investigations led directly into the protein → nucleic acid sequence now recognized for modern cells (cf. also Dillon, 1978). The

protein → nucleic acid sequence is thus part of the protobiology → biology sequence.

A coding mechanism from proteins-first

The widely held view of non-enzymic 'zippering' of new bases stacked on already formed polynucleotides in modern cells has been dominant. This has undoubtedly been at least partly responsible for the transposed idea (e.g. Ponnamperuma, 1968) that a nucleic acid coding mechanism existed in the first cells. The physical models of protobiology, however, could not fit such a pattern; they led to the most critical questioning of the DNA-first assumption, as we have seen. What has become clear more recently (Commoner, 1968; Kornberg, 1980) is that the idea of the primacy of naked DNA in the *modern* biochemical economy is also incorrect, in line with Kornberg's critique of the problem. To recognize that DNA alone is not capable of carrying the burden of inheritance is certainly not to say, however, that it does not play a key role. It represents the blueprint through which the specifications of the living cell are bequeathed to the next generation.

How did evolution first incorporate DNA into its scheme of reproduction? A considerable part of this question is answered by experiment. First, however, it is necessary to examine the view which is forceful merely by virtue of the fact that it has been taught to numerous generations of students. This is the sophistry of 'DNA self-replication'.

No evidence exists that DNA replicates itself. All that has been shown is that DNA is replicated. DNA is thus passive, the result of transitive actions. No shred of evidence exists to demonstrate that DNA can 'make' anything, let alone more of itself. Approximately a century of biochemical investigation has taught us that the agents of manufacture in the cell are protein enzymes. Moreover, they do not operate alone. They require energy-rich substances, which are predominantly the ubiquitous ATP, occasionally GTP, and some other special compounds.

The phrase 'DNA self-replication', even though sometimes qualified in part, has grown in use (Watson, 1976, p.213; Luria, 1973; Eigen *et al.*, 1981). Most interesting is the fact that self-replication as a term is typically used in publications that explicitly refer also to the enzymes that carry out the processes (e.g. Eigen *et al.*, 1981). The difference in outlook is not an expression of intellectual territoriality. It seems, however, that only those students who were biochemically educated and have been exposed to the vast wonderful world of enzymes and all that they do, e.g. Kornberg (1980), acknowledge an as yet incompletely defined role of enzymes in inheritance.

If we recognize that DNA is made by enzyme proteins (Commoner, 1968;

Dillon, 1978; Kornberg, 1980) the question that properly concerns us here is whether it can be shown that the concept of proteins-first will lead into the mechanism of modern genetic coding.

This possibility has so far been recognized in mechanistic detail in only one way. The new perspective is the result of experiments in protobiochemistry. The first recognition of the possibility stemmed from the finding that a lysine-rich proteinoid catalyzes each of the two principal syntheses of biomacromolecules: the synthesis of peptide bonds and the synthesis of internucleotide bonds (Fox *et al.*, 1974). With the assumption that all lysine-rich peptides can synthesize peptides from all kinds of amino acid (Nakashima and Fox, 1980; Fox and Nakashima, 1980), a mechanism for the origin of the genetic code within cells resulted (Fox, 1981c,d).

The synthetic activity is found in thermal peptides rich (ca. 20–25 per cent) in any basic amino acid (Syren and Fox, 1982). Such activity is observed in aqueous solution, in phase-separated particles composed of basic and acidic proteinoids, and in phase-separated particles composed of basic proteinoid and polynucleotide. Laboratory protocells composed of basic and acidic proteinoids are stable at pHs above 7·0 (Fox and Yuyama, 1963b; Snyder and Fox, 1975) and are the kind that have been artificially fossilized (Francis *et al.*, 1978). They are also catalytically active (Fox and Nakashima, 1980). Their catalytic activity is due to basic proteinoid contained in the particles. This locale of the evolving cell itself was an ideal setting for the origins and evolution of the genetic code. The experimental findings thus bridge the component protobiological phenomena to what looks to be the first biological locale for the origin of the genetic mechanism, in which nucleic acid and protein syntheses were necessarily intertwined. Such direct interactions would then have evolved to mechanisms involving synthetases, adaptors, etc.

Self-ordering and self-organizing

Several statements have been made about the critical nature of self-organization in evolution (Fox, 1960; Matsuno, 1982; Nicolis and Prigogine, 1977; Eigen, 1971; Fox and Matsuno, 1983). The interdigitation of self-organization with other processes and within processes merits some consideration.

Self-sequencing is self-organizing

The significance of self-organization has been recognized by numerous evolutionists (Pasteur in Vallery-Radot, 1922; Fox, 1960; Eigen, 1971a,b; Nicolis and Prigogine, 1977). The reality of self-organization has been challenged by Yockey (1981) in a paper peppered with allusions to scripture

and suffering from failure to recognize that modern living systems are not primordial systems. Among those who recognize the significance of self-organization either to early steps in evolution or to biochemical steps in modern organisms, some (Eigen, 1971a,b; Crick, 1981) have failed to allow that self-sequencing of amino acids was an obligatory early step in self-organization. This is perhaps due to a basic commitment to the pervasive concept of the random matrix. The recognition of the larger process of self-organization was belated, requiring many decades since a definition of the problem by Pasteur in 1864 (Vallery-Radot, 1922), so it will not be surprising if general awareness of the principle of self-sequencing will need more time.

The evolutionary descendant of self-ordering is enzyme specificity

Writers such as Calvin (1969) and Dillon (1978) have discussed the phenomenon of self-sequencing (self-ordering) of amino acids in the primordial context. They have stated in addition that modern relicts of this phenomenon must be available.

Such a relict has been proposed (Fox, 1981c) to be the specificity of all evolved enzymes. The original self-ordering of amino acids is explained as due to the steric relationships of the various amino acids (Fox and Dose, 1977). This would largely be an expression of amino acid sidechains (Fox, 1980a). When these same amino acids became fixed in proteinoids, and later in proteins, the sidechains again supplied steric information for reactions with substrates in metabolism. According to this view, the relict of self-ordering is a very pervasive one, i.e. the specificities of all enzyme-substrate interactions.

Self-ordering is self-limiting

The significance of a self-limited array is that it is non-random, and can be very highly so. The difficulties of accomodating evolutionary fact to a random matrix has been commented on. The recognition of self-ordering as a key early step in evolution has allowed a quantitative valuation to be placed on dynamic non-randomness (Nakashima *et al.*, 1977).

The couching of proteinoid evolution within cosmic evolution

The concept protobiology → biology suggests in turn that this transition fits into a larger transitional sequence. Especially has there been the need to understand how materials of the first cells could have become more ordered than those protocells. The experimental evidence for much non-randomness in both protocells and proteinoids has been accumulating for over twenty years. Much more recently, data from the Big Bang temperature studies

have appeared (Davies, 1978). The evidence on events within the first 24 hours of the Big Bang suggests that the initial Universe (Eriksson *et al.*, 1982) was highly non-random. An earlier similar suggestion was that of Sakharov (1966), who inferred internal constraints in an early expanding universe.

When that picture is placed in tandem with the inferences on order in cellular precursors, and then in cells, the sequence appears to be a cosmic reaffirmation of the second law of thermodynamics (Fox, 1980b). In this perspective, a smooth transition of protobiology → biology, while still lacking much interstitial detail, is more easily defended. The relationship between order between galaxies and between molecules still needs to be spelled out, however.

The significance of the proteinoid evolutionary sequence is that it has revealed (a) the crucial nature of self-sequencing of amino acids to give non-random informed polymers, to yield (b) a temporally primary and dynamic proteinoid protocell and (c) the remarkable (Rutten, 1971; Price, 1974; Lehninger, 1975; Florkin, 1975) manifold (proto)biological functions of that unit of protolife (Fox, 1978). This sequence is rooted in the non-randomness of matter in the prebiotic era, both astronomical and geological.

Time-based instructive evolution of macromolecules

In two papers, Wassermann (1982a,b) has discussed principal consequences of recognizing internal, or molecular, selection relative to Darwin's natural selection. Wassermann uses the term molecular selection, which we had employed earlier (Fox and Dose, 1977, p.261) as a synonym for self-ordering or self-sequencing (in the case of amino acids). Wassermann points out that TIMA (Time-based Instructive Evolution of Macromolecules) 'would have an overwhelming advantage over the neo-Darwinian idea of exclusive evolution by environmental selection combined with random genesis of molecules. "Molecular selection" is an active selection which can dispense with the assumed highly improbable random neo-Darwinian origin of macromolecules'.

Wassermann relates TIMA and molecular selection to other topics such as DNA, RNA, germ-line cell genomes, and punctuated equilibria.

THE POPULAR AND PROTEINOID PARADIGMS

Matrix: random or non-random?

Most of the principal aspects in which the proteinoid theory differs from the popular paradigm are listed in Table 2·5. The tenets formulated from data

TABLE 2·5 Popular and proteinoid paradigms

Popular paradigm inferred from modern cells	*Proteinoid paradigm inferred from experiments*
Random matrix	Non-random matrix
Random polymerization of amino acids	Non-random polymerization of amino acids
Order out of chaos	Chaos out of order
Chance variation (outside)	Orthogenetic variation (endogenous)
Long prebiotic evolution	Brief prebiotic evolution (< 12h)
Intricate processes of origin	Simple process of origin
Replication at the molecular level first	Replication at the cellular level first
Enzyme-free replication of DNA	Enzymic replication of DNA
Emergence of cells after the inheritance mechanism (DNA-first)	Emergence of inheritance mechanism in cells (Protein-first)

from protobiological simulation are obtained only from proteinoid since, evidently, that is the only approach to have retraced the steps in molecular evolution. In the popular paradigm the neo-Darwinian premise is prominent and fundamental.

The random variation of neo-Darwinism begins from, and would indeed require, a random matrix. Almost without exception, leaders in the development of newer *gedanken* on the origin of life (Monod, 1971; Eigen, 1971a,b; Luria, 1973; Eigen and Schuster, 1979; Crick, 1981) build their concepts on the 'armchair' assumption of a random matrix.

In contrast, the proteinoid paradigm is based on a non-random matrix that functioned as the staging area for organic evolution. In this experimentally derived paradigm, the principal type of solid matter in the cell, structurally and functionally, was protein. As has been explained, the first protein was proteinoid, or 'thermal protein' as it is listed in Chemical Abstracts indexes.

The popular paradigm, as explained, identifies the first informational biomacromolecules as nucleic acid. However, the proponents of this view have not explained how the nucleic acid came into existence, nor how it could have come into existence without the agency or direction of prior specific protein. Nor have any of the proponents explained how nucleic acids could have been replicated without prior specific proteins.

The usual assumption for the origin of nucleic acid is that it arose from a random matrix by chance (*see* Introduction). According to the experiments, however, the proteins arose in a determinate and endogenous fashion, not by chance and not by the action of outside agents. *The matrix was non-random.* It was non-random because the proteins obtained instructions from the reactant amino acids.