COMMENTARY

SYNTHESIS OF LIFE IN THE LAB? DEFINING A PROTOLIVING SYSTEM

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SALTHE'S REVIEW (1989) of my book, *The Emergence of Life: Darwinian Evolution from the Inside*, has raised anew the question of whether life has been synthesized in the lab. Salthe states that the book ". . . verges on implying that, without knowing it at the time, his group synthesized life in the lab. . . . but almost no one would say that, without genes, [microspheres] are alive." My acquaintance with many similar comments has prompted the present analysis of what is obviously a source of perplexity. The perplexity may exist in large part because of a shifting paradigm. The difficulty is related, for example, to awareness of the need for participation in the specifics of life by RNA, which is seen on close analysis, however, as requiring a "simpler" precursor (Waldrop, 1989).

The difficulty appears to derive also from the fact that, on one hand, a laboratory-assembled organism is being used for specification, whereas a fully modern organism is used by others as the standard of biofunctionality. Most students are necessarily less familiar with the findings in forward-directed evolutionary experiments (Fox, 1988b) than with existing organisms. The laboratory organism (proteinoid microsphere) in question has arisen under presumably primitive conditions, since the thermal protein from which it is assembled is made by the seemingly crude geological heating of mixtures of amino acids. Historically, the first finding of self-organization from such precursor protein to a cell (Fox et al., 1959) is also a process of maximal simplicity. These are processes disarmingly more direct than the exceedingly complex ATP-energized set of reactions required for the synthesis of modern proteins. On the other hand, the answer strains conventional thinking, because it spans more than three billion years.

The "synthesis" of an organism in the lab holds special significance as an affirmation of cell analyses. This relationship and conceptual need has been explained by Young (1984). Such verification is an extension to cellular science from organic chemistry (Fox, 1975) in which synthesis of bioorganic compounds is a traditional confirmation of the analysis by organic chemists of the chemical structure of a natural compound.

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Comments by others than Salthe

Since at least as long ago as 1972, critics other than Salthe have raised the question of whether the proteinoid microsphere (Fox, 1988a) is a living system synthesized in the laboratory. These comments are generally in a context that recognizes the positive advances. The question is then typically followed with an answer by the author that the unit cannot be regarded as alive, essentially because it contains no genes, DNA, or RNA (cf. Fox, 1988b). In 1972, Chemical Abstracts saw fit to index proteinoid under "protein," subheading "thermal." By the middle 1980s growing lists of primitive biofunctional properties had been accumulated for the thermal proteins as polymers and for the proteinoid microspheres as assembled cellular structures. From integration of these data, two inferences seem justified: (a) the proteinoid microsphere is *not* a modern organism; (b) this laboratory protoorganism is the only representative we have of a (*proto*) living organism, as had been proposed in 1972 (Fox and Dose). The fundamental question of where this evolutionary succession derived its first biological information had been answered by the process of the self-sequencing of amino acids; this information-generating system was shown experimentally to be capable of transferring information through informed polypeptides into cellular polynucleotides.

The criticisms of nucleic acid-less organisms are exemplified by Sylvester-Bradley (1973), who reviewed the first edition of *Molecular Evolution and the Origin of Life*, by Fox and Dose (1972), as follows:

> Fox and Dose are in danger of creating the mistaken impression that their protocells are almost already 'alive.' The student who defines life in functional terms may be perplexed, for in the laboratory these protocells absorb food from the environment, grow, and multiply by fission. Is this not life? The answer is, of course, very firmly in the negative, . . . and we still await experiments in which protocells have been persuaded to incorporate nucleic acids.

Among the long list of experimental novelties recited in the above book, page 229 presents photographs of synthetic organelles incorporating DNA or RNA which had easily been "persuaded" to combine with proteinoid rich in lysine content. Since 1972, in addition, there have been recorded the synthesis of polyribonucleotides from monoribonucleotides by lysine-rich proteinoid in microsphere suspensions (Jungck and Fox, 1973), and proteinoids that had already been shown to react selectively with enzymatically synthesized polyribonucleotides in which information was transferred in either direction (Yuki and Fox, 1969). A primary point, however, is that while absence of nucleic acid was taken in a review to signify that the organism was disqualified from being alive, the quality designated as missing had already been found and displayed.

In a third example, from Kleinsmith and Kish (1988, p. 28), whose interpretations are closely linked to experimental results, one reads:

> Microspheres are no more than small vesicles containing mediocre catalysts and therefore capable of a primitive type of metabolism, growth and division. Microspheres . . . have no genetic system for encoding and accurately transmitting hereditary information. Of course there is no reason for believing that the first cells arising on earth had to resemble those occurring today, so the term **protocell** has been introduced to refer to a primitive cell-like structure that might have been the evolutionary precursor of contemporary cells. Microspheres are certainly a plausible model for such protocells.

The multibillion year flowsheet from self-sequencing of heated amino acids to modern cells serves as a clean and continuous answer to the classical chicken-egg question of which came first: nucleic acid or protein? A summary of the argument, including those quoted, recognizes that the proteinoid microsphere possesses the classical properties of (a) metabolism (to an incompletely developed degree but including synthetic capability for peptides and oligonucleotides; see also Wessells and Hopson, 1988, p. 443), (b) growth (to a programmed range of size), (c) reproduction (by accretive growth), and other properties (Fox, 1980, 1989a). The microsphere does not have

a contemporary nucleic acid genetic coding system, but the cell has retained through three billion years a plausible evolutionary precursor in the mechanism of self-sequencing of amino acids (Ivanov and Förtsch, 1986). This mechanism is a manifestation of molecules and to it is ascribed a "pregenetic memory"; the memory is in the molecules. When the microspheres are regarded as synthetic protoorganisms, a large part of evolution is visualized as having occurred before descendants of the first proteinoid microspheres became modern organisms.

The fact that a single agent, lysine-rich proteinoid, catalyzes the synthesis of peptides from amino acids and also catalyzes the formation of polynucleotides from mononucleotides in the same cellular locale (Fox, 1981) provides a first explanation for the origin of the genetic coding mechanism. Such products would be the first templated cells.

Nonrandomness

The assumptions about the need for prebiotic or protobiotic nucleic acids, as voiced by cited and uncited authors, interdigitate with another assumption, that of prebiotic randomness. In modern science this view (Fox and Windsor, 1984) has been put forth by such workers as Crick, Monod, Prigogine, and Eigen, although Eigen (1986) has recently spoken for nonrandomness. The conceptual need for early evolutionary action of polynucleotides has itself been linked to a third assumption: that ancient synthesis of protein would have been disorderly (Oparin, 1957), and that life results from "order out of chaos" (Prigogine and Stengers, 1984).

Recognition of the nonrandomness of thermal protein synthesis prior to a genetic coding mechanism has explained the laboratory synthesis of the protoorganism described herein; it was composed of already ordered proteins, as the results reveal (Fox, 1988a, 1989b). This nonrandomness was reported for thermal polycondensation of mixed amino acids in a melt (Fox, 1988a) and for aqueous polycondensation of mixed aminoacyl adenylates synthesized by thermal proteins, which were themselves necessarily nonrandom, as indicated herein (Nakashima and Fox, 1972).

Where is evidence for nucleic acids first?

The finding of nonrandomness has recently been supported and extended by Tyagi and Ponnamperuma (1990) in an elegant experimental examination. They showed that specificities stem from amino acid residues of the reacting aminoacyl adenylates, but not from the ribomononucleotides. These authors emphasize the extent to which the assumption that peptides produced without instruction from polynucleotides are expected to have been random ones; they cite Crick, Eigen and Schuster, Cairns-Smith, Dyson, and Weiner and Maizels. Prigogine and Stengers (1984) and de Duve (1988), in addition, reason from a premise of randomness. As stated earlier, however, Eigen has departed from that position, and de Duve has reasoned from reverse translation, a process which is seen on analysis to require ordered proteins before ordered polynucleotide residues.

The tenet of randomness in protein before life originated has been negated in all relevant experimental results, beginning with the thermal self-sequencing of amino acids and continuing in evolution well beyond that stage. The concept of randomness has been characterized as a source of conceptual difficulty for nearly a full forty years (Fox, 1989) in which the emergence of life has been traced—except, of course, in the earlier pioneering retracement experiments of Herrera (1942).

From ancient protein synthesis to modern protein synthesis

Supporting and extending, as well as partly suggesting, a 3 billion year-plus evolution is the finding by Ivanov and Förtsch (1986) that the self-sequencing mechanism has been evolutionarily conserved since its inception. The Ivanov evidence was accumulated from statistical studies of amino acid residue sequences cataloged for 2898 modern proteins. In addition to the chemical evidence for initial endogenously limited polymerization, the accumulation of evidence for biofunc-

tional properties in the proteinoids and microspheres (Florkin, 1975; Fox, 1980, 1989a) provide the evidence for how the biological realm emerged from the chemical realm. Ivanov and Förtsch also indicate how processes in the protobiological realm evolved to the modern realm through a protein-synthesis mechanism.

Relative to the reverse translation mechanism discussed by de Duve, the results of Tyagi and Ponnamperuma (1990) support and extend the earlier experimental results indicating that information can flow in either direction in unevolved systems (Yuki and Fox, 1969). The results of Ivanov and Förtsch establish a connection between ancient synthesis and modern synthesis that affects the interpretations of Tyagi and Ponnamperuma. The interamino acid effects were shown also by Ivanov and Förtsch to extend through more residues than the nearest-neighbor amino acid residues postulated by Tyagi and Ponnamperuma.

Summary

The synthesis of a living system in the lab has been judged by a number of critics as partly attained by the proteinoid microsphere because of its primitive properties of metabolism, growth, and reproduction. These same critics, however, judge the organism as not alive, or as being 50 to 75 percent alive (Baltscheffsky and Jurka, 1984), owing to the absence of a nucleic acid genetic coding mechanism. The experiments in retracing evolution suggest, however, that the self-sequencing of amino acids was the evolutionary precursor of modern nucleic acid templating; the genetic memory is the molecule. The proteinoid microsphere is *not* a modern living system, but does represent at least a protoliving system (Fox and Dose, 1972). Berra (1990, p. 75) has commented on other difficulties in defining a protoliving system. In Berra's opinion, metabolism, reproduction, responsiveness to stimuli, and cellularity constitute or describe aliveness. These properties characterize proteinoid microspheres.

A number of experiments demonstrate that amino acids in aminoacyl adenylates yield specific products, whereas nucleotides are without effect. For this and related reasons, especially the demonstrated self-sequencing of amino acids when they are warmed, resultant biofunctional properties of self-assembled microstructures, and demonstrated self-sequencing of amino acids in modern systems, the results appear to bridge from the chemical era to the biological period.

All the above emerges from a departure in style of research (Young, 1984; Pauling and Zuckerkandl, 1972). The latter authors said, "It appears likely that biogenesis is the passage from a 'non-living system' existing in a large number of states to a 'living' system also existing in a large number of states." In this wider evolutionary view one finds room for both the protocell and the modern cell, each type having derived its information from the earliest stage of amino acid self-sequencing (Ivanov and Förtsch, 1986; Fox, 1988a).

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