# FROM PROTEINOID MICROSPHERE TO CONTEMPORARY CELL: FORMATION OF INTERNUCLEOTIDE AND PEPTIDE BONDS BY PROTEINOID PARTICLES

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**Abstract.** Proteinoid microspheres of appropriate sorts promote the conversion of ATP to adenine dinucleotide and adenine trinucleotide. Other microparticles composed of basic proteinoid and enzymically synthesized poly A cause the conversion of ATP and phenylalanine to various peptides of phenylalanine. When viewed in a context with the origin and properties of proteinoid microspheres, these results model the origin from a protocell of a more contemporary type of cell able to synthesize its own polyamino acids and polynucleotides. Related earlier experiments explain in part the origin of the genetic code and mechanism.

Few societies are organized for the study of a single problem, such as that of the origin of life. In the same vein of thought, perhaps no problem has been so thoroughly explored by hypotheses prior to experimentation as has this one. Accordingly, no experimental model can yield results consistent with more than a fraction of the hypotheses that have been so abundant, especially for the emergence of macromolecules and cells. The proteinoid microsphere, as a laboratory model for the replicating protocell (Fox and Dose, 1972; Fox, 1973a) reflects concepts of a primordial sequence (Fox, 1971) as those are implicit in the expressed thought of a number of authors. Among the contributors whose ideas can be interpreted as consonant with that of a cell initiating protein and nucleic acid synthesis and Darwinian selection have been Wald (1954), Van Niel (1956), Oparin (1957), Lederberg (1959), Ehrensvard (1960–1962), and Prosser (1970). The integrated view from their hypotheses and from our experimental results is expressed concisely by the sequence: *protoprotein* → heterotrophic *protocell* → macromolecule-synthesizing *cell.*

The contributions of the above-named authors (and others) have been almost entirely theoretical. An integrated conceptualization benefitted from support by relevant experiments and it was extended by additional concepts that emerged almost solely from experiments. Two of the salient inferences derived from the experiments were (a) the 'self'-ordering properties of reacting amino acids (Dose and Rauchfuss, 1972) and (b) the onset of reproduction as one function associated with others in the proteinoid microsystems assembled from the polymers resulting from the 'self'-ordering reactions (Fox and Dose, 1972). In fact, all that has been learned of this system, including (b), stems from the first experiment based on concept (a) (Fox, 1973a). The experiments of the Oparin school with coacervate droplets have explained the need for an early appearance of boundaried microparticles (Oparin, 1966). The coacervate droplets of Oparin have demonstrated utility in the protection of cellular contents, in the promotion of intracellular reaction sequences, and in favoring of a concentra-

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tion of growing polymers. Van Niel (1956) has exemplified the combined viewpoints of those who favor the concept of an early minimal cell by stating, "Acceptance of the postulate that chemical evolution preceded biopoesis further suggests that the organized structures representing primitive life were capable of self-reproduction before they acquired mechanisms by means of which they could chemically transform the components of their environment." In 1967, we reported the evidence that proteinoid microspheres are organized structures capable of 'self'-reproduction (Fox *et al.*, 1967; Lehninger, 1970; Szent Gyoergyi, 1972).

In this paper, we report mechanisms by which two kinds of microparticle from proteinoid use *one* component of their environment, ATP, to produce *two* kinds of polymer molecule, polyamino acids and polynucleotides. We have been able to find polymers of amino acids formed by, and oligomers of adenylic acid formed from, the same reactant, ATP. The fact that aminoacyl adenylates (products of amino acids and ATP) contain both amino acid and adenylic acid monomers has provided a basis for understanding how both *cellular* proteins and nucleic acids would have arisen in an almost simultaneous relationship (Nakashima *et al.*, 1970). Ehrensvärd predicted with some accuracy the primacy of enzymically active protein and the emergence of nucleic acids, as modelled (Fox and Dose, 1972) when he stated, "The stabilizers in cells today in the form of genes in chromosome bodies should have originated as *by-products* of early chemical activity in loosely organized units of catalysts" (Ehrensvärd, 1962).

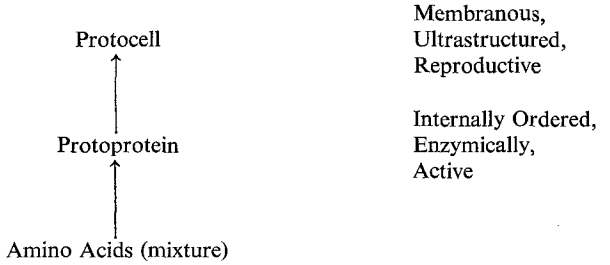


Fig. 1. Properties of protoprotein and protocell models derived from mixed amino acids (Fox and Dose, 1972).

Figure 1 presents some salient aspects of the origin of proteinoid microspheres. As explained elsewhere, evidence has been presented that these microunits are reproductive, evolvable (Fox and Dose, 1972), and heritable (Hsu *et al.*, 1971). All of these properties are derivative of the basic ability to replicate at the systems level, first shown in one way (*cf.* Fox, 1973b) in 1967 (Fox *et al.*, 1967). The systems level, however, is the level at which Darwinian selection operates (Prosser, 1970).

The emergence of the contemporary cell demands two large consecutive steps: (a) the origin of the protocell and (b) an evolution to the contemporary cell. Our understanding of the properties of the protocell permits us to subtract those functions from those of the contemporary cell. When we do, we find that what was needed in step

(b) was the emergence of cellular syntheses of both protein and nucleic acids in a coded relationship, coordinated with a mechanism for the energizing of synthesis of anhydropolymers. One purpose of this paper is to describe recent experimental progress toward this conceptual objective.

Figure 2a demonstrates that the addition of ATP to appropriate basic proteinoid in solution of magnesium chloride yields adenine dinucleotide. This result is a signpost to concepts of the origin of proto RNA polymerase. It adds to the now long list of enzymelike properties in proteinoids (Dose, 1971) the catalysis of a synthetic reaction (ATP-energized).

Figure 2b presents the result when ATP is added to microspheres composed of

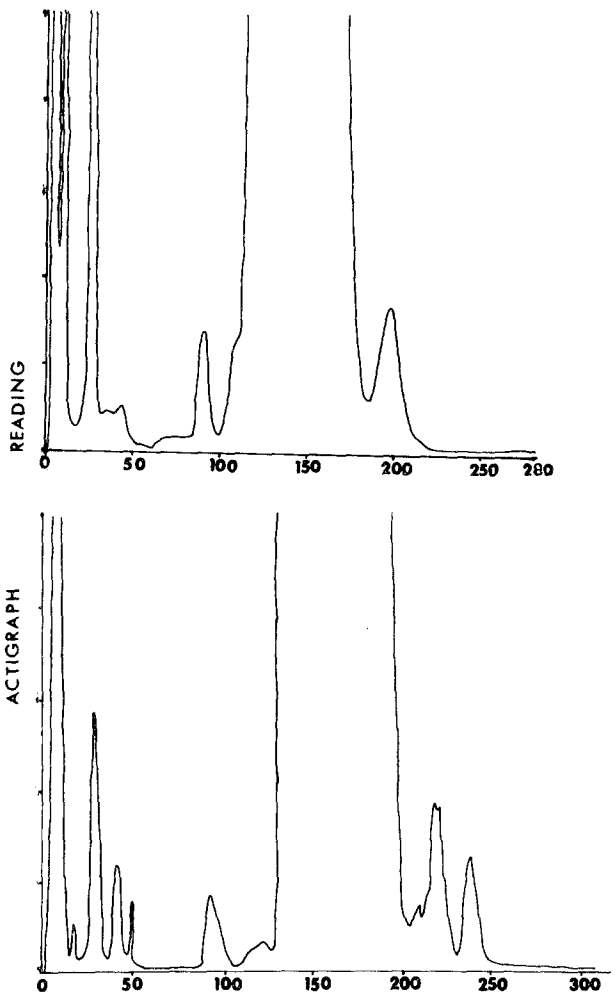


Fig. 2a–b. (a) Formation of adenine dinucleotide in 20 mM $MgCl_2$ solution. Rightmost peak is adenine dinucleotide. (b) Formation of adenine dinucleotide and adenine trinucleotide by microspheres of basic and acidic proteinoids suspended in 20 mM $MgCl_2$ solution. Rightmost peaks are adenine dinucleotide and adenine trinucleotide, from left to right respectively.

acidic and basic proteinoids. In this case a significant fraction of the product is adenine trinucleotide.

The above products were obtained by mere incubation of U-$^{14}$C-ATP for 24 hr at 37°. These products were fractionated on DEAE-cellulose, and monitored by radioactivity and by UV absorption.

Table I illustrates the results from comparing the reactions of ATP in aqueous solution alone, in the presence of basic proteinoid, in the presence of microspheres of acidic proteinoid, and in the presence of microspheres composed of acidic and basic proteinoids.

TABLE I

Yields of adenine dinucleotide and trinucleotide in various systems with ATP (at 37°, 2 days)

| Products | Reactants | | | |
|---|---|---|---|---|
| | ATP in aqueous solution | ATP with basic proteinoid | ATP with acidic ptd microspheres | ATP with acidic-basic ptd microspheres |
| Adenine, adenosine, cAMP, AMP, ADP | 12.6% | 14.7% | 14.8% | 13.0% |
| ATP recovered | 86.8 | 82.9 | 82.9 | 84.7 |
| Oligo A eluting beyond ATP | 0.7 | 2.2 | 2.2 | 2.3 |
| $\frac{\text{Trinucleotide}}{\text{Dinucleotide}}$ | 0.0 | 0.0 | 0.2 | 0.5 |

The identity of the di- and tri-nucleotides was confirmed by chromatography on DEAE-cellulose followed by recognition of radioactive product and by UV, by thin-layer chromatography in two systems, and by nucleotide/nucleoside ratios after treatment with alkaline phosphatase and hydrolysis (Junck and Fox, 1973). It is however notable that a definite peak for the trinucleotide has been found only in the microsphere systems, under conditions that do not yield adenine trinucleotide in the absence such particles.

The possibility of formation of polymers larger than trinucleotides both in solution and in a cell-like structure is being investigated. The trinucleotide is already, however, a minimal coding unit (Nirenberg, 1967), perhaps sufficient in size and stability to have initiated a polynucleotide-dependent cellular evolution.

These experimental results are consistent with the view that nucleic acids first appeared in evolution subsequent to cells and their precursor protoprotein, which had also some catalytic activities (Fox and Dose, 1972). This is the sequence of events expressed by Van Niel and by Ehrensvärd, as stated earlier.

The demonstration that adenylic acid is most effectively polymerized in the locale of a microsystem is consistent with thermodynamic reasoning, which indicates that the formation of anhydropolymers is favored under either geological hypohydrous conditions or in hypohydrous zones in cells (Fox and Dose, 1972). The origin of ATP as an energy-rich reactant has been largely modelled (Ryan and Fox, 1973).

Experiments suggesting the origins of cellular protein have employed another kind of microparticle. These particles are models for protoribosomes; they are produced from polynucleotides and basic proteinoids (Waehneldt and Fox, 1968).

When enzymically synthesized poly A is allowed to form particles with lysine-rich proteinoid (37% lysine), microparticles result. When ATP, metal cations, and U-$^{14}$C-phenylalanine are added to these particles, the phenylalanine is polymerized.

Numerous experiments with cations $Na^+$, $K^+$, $Ca^{++}$, and $Mg^{++}$ and combinations of those cations showed that ATP functions best in combination with $K^+$ and $Mg^{++}$, which are significant in contemporary protein biosynthesis. When the microparticles are also added, significant yields of di-phe (III), triphe (IV), and a higher polymer of

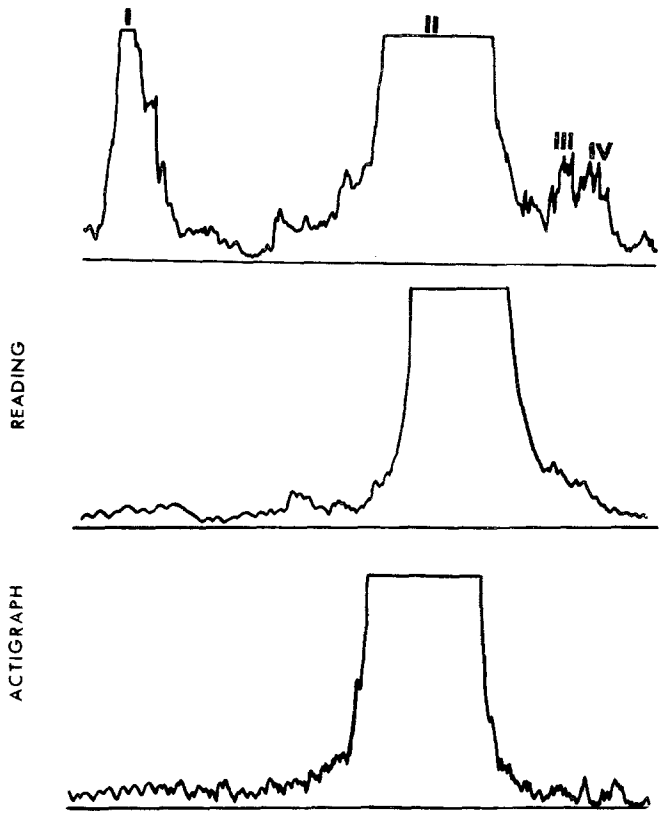


Fig. 3a–b. (a) Production of polyphenylalanines by particles composed of lysinerich proteinoid and poly A. I has *phe/N-*phe = 11 but is associated with nonphe material, II is *phe, III is *phe-*phe, IV is *phe-*phe-*phe. (b) Same as (a), without ATP. (c) Same as (a), without particles.

phe (I) are obtained (Figure 3). Figures 3a and 3b reveal that ATP is necessary to energize the synthesis, since 3b is the same as 3a without ATP. Without the microparticles, but with ATP and $K^+$ and $Mg^{++}$, no peptides result from phe, as Figure 3c indicates. Lysine-rich proteinoid alone, instead of microparticles, has almost no activity. Activation by ATP, and hypohydrous or surface conditions provided by an extrasolution particle are thus both necessary to the peptides synthesized.

Analysis of the products of Figure 3a provides information on their identities. Peak III corresponds by $R_f$ to phe-phe-phe. Its identity was confirmed by conversion to its DNP derivative and comparison chromatography with authentic DNP-phe-phe-phe in a solvent of toluene: pyridine: 2-chloroethanol: $H_2O$: $NH_4OH$.

Fraction I in Figure 3a proved to have specifications of a higher peptide or peptide mixture. It has been dinitrophenylated, and the DNP derivatives chromatographed on paper with the toluene solvent. When hydrolyzed, the products yield Figure 4. The DNPphe is obtained by extracting the hydrolyzate with diethyl ether and chromatographing the solid obtained on evaporation; the phe in Figure 4 is obtained by chromatography also. When correction is made for the partial hydrolysis of DNPphe during its release from DNP-I (70% recovery), the ratio of phe/N-phe is calculated to be 11/1. Fraction I or its DNP derivative are revealed by gel exclusion studies to be of high molecular weight; perhaps some proteinoid or AMP is closely adherent. The phe/N-phe ratio however provides an accurate measure of the size of the peptide part of the material in this peak since phe is the only radioactive moiety, and each component is measured by its radioactivity.

The total yield tends to fall into the range of 30–40 $\mu\mu$moles of phenylalanine incorporated into peptides per mg of poly A in the particles. In the first case to come to hand from the literature of molecular biology after this result was obtained, fig fruit ribosomes were found to incorporate 31.8 $\mu\mu$moles of phe per mg of RNA (Marii *et al.*, 1972).

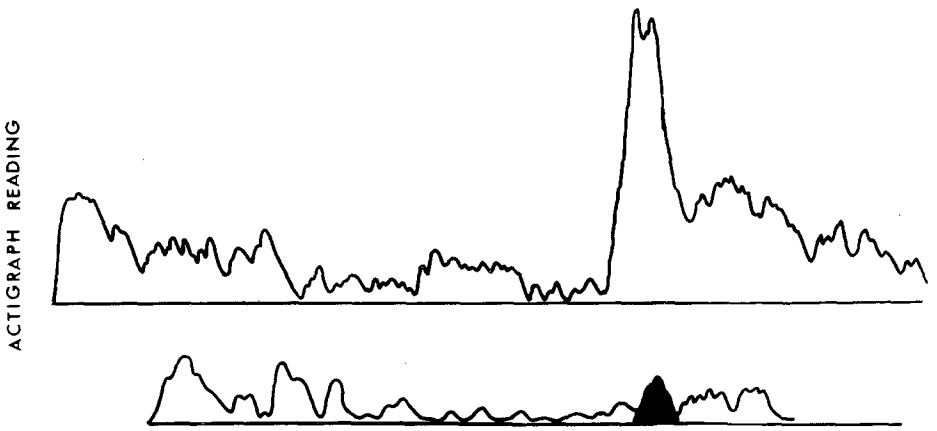


Fig. 4. Radiochromatograms of *phe and DNP*phe from Fraction I of Figure 3a; after hydrolysis. Tall peak in upper chromatogram is *phe. Other peaks may be *phe complexed to fragments of slowly hydrolyzable lysinerich proteinoid. DNP-*Phe in lower chromatogram is dark peak (yellow), after DNPylation of I and subsequent hydrolysis of DNP-I.

The models for protoribosomes, which we call *protosomes*, exhibit a lifetime of a few hours. Figure 5 demonstrates how the peptide-synthesizing activity rises rapidly to a maximum and then falls off with time of incubation at 25°. The need for an early cell, either to protect protoribosomes or to contain their continuous generation, can accordingly be visualized.

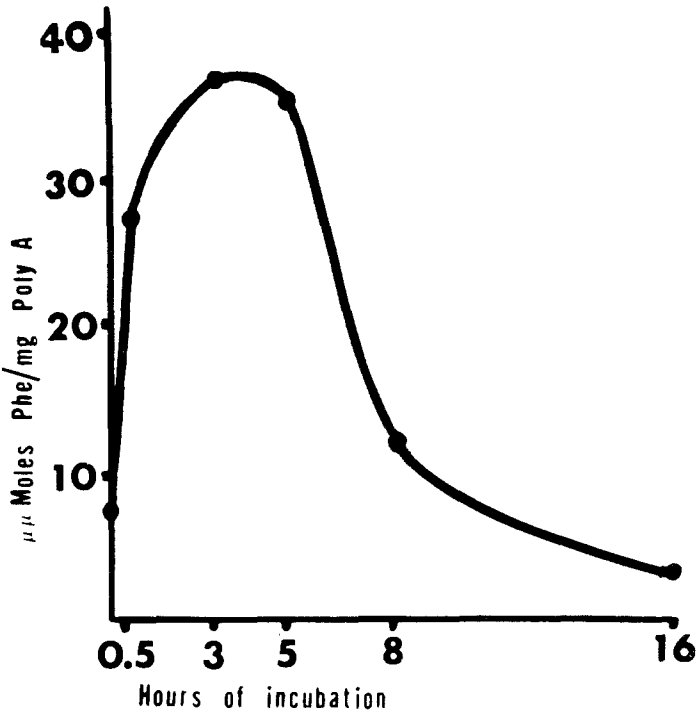


Fig. 5. Rise and decay with time of poly A-lysinerich proteinoid in polymerization of phenylalanine.

The new experiments just described begin to exaplain in part how protein biosynthesis and nucleic acid biosynthesis could have originated in replicating protocells through a contemporary energy-transfer substance, ATP. A number of related questions deserve attention in this context. These include the origin of ATP, the nature of models for protoribosomes, the possibility of selective reactions in the formation and action of such microparticles, the possibility of codonicity in simple systems, and questions of reversibility of direction of recognition from protoproteins to protonucleic acids and protonucleic acids to protoproteins. Data from earlier experimental demonstrations bear on all of these questions.

The common factor in the work on all but one of these questions is the basic lysine-rich proteinoid. Waehneldt and Fox showed in 1968 that proteinoids containing sufficient proportions of lysine would interact with RNA or DNA to yield particulate complexes (Figure 6). These nucleoproteinoid complexes exhibit salt-sensitivity and pH-sensitivity comparable to that of nucleoproteinaceous organelles.

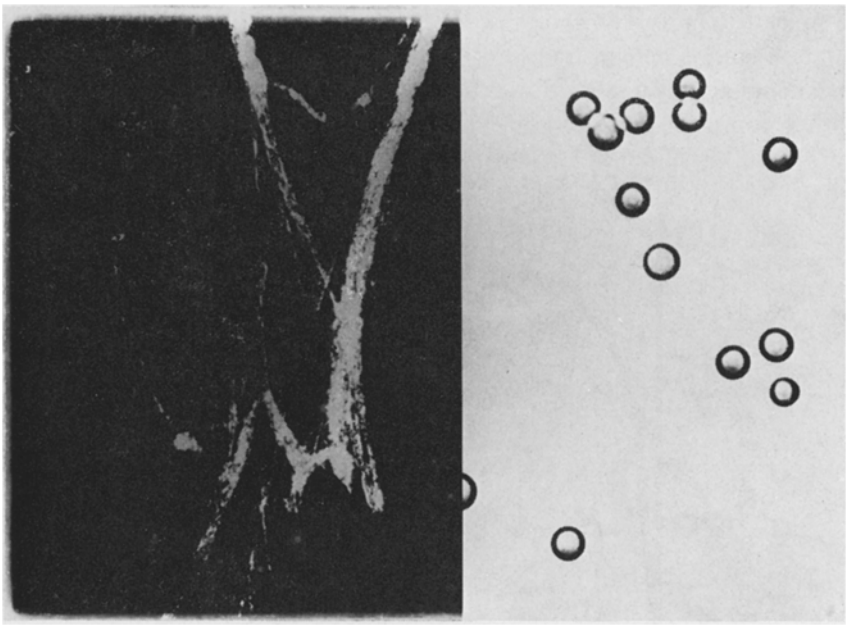

Fig. 6. Microfibers from calf thymus DNA and lysinerich proteinoid, and microglobules from yeast RNA and the same lysinerich proteinoid.

When the nucleic acids were substituted in such experiments by enzymically synthesized homopolynucleotides, Yuki observed that the interaction was selective, and that each kind of macromolecule could recognize the other (Yuki and Fox, 1969). Some data are presented in Table II.

These selective interactions to form microparticles are a kind of result that was not forecast by numerous attempts to observe interactions of polynucleotides with amino acids (Kenyon, 1973; Fox and Dose, 1972). The interaction of polynucleotides with polyamino acids is, however, demonstrable and selective, and this was first shown with

TABLE II

Selective interactions between two basic proteinoids and five homopolyribonucleotides to form microparticles (adapted from Yuki and Fox, 1969)

| Polynucleotide | Lysine-rich, arginine-free proteinoid | Arginine-rich, lysine-free proteinoid |
|---|---|---|
| Poly C | +++ | 0 |
| Poly U | + | + |
| Poly A | 0 | + |
| Poly G | 0 | +++ |
| Poly I | 0 | +++ |

○ = no turbidity, + = some turbidity, +++ = much turbidity

synthetic macromolecules in this research. The strong interactions of polyamino acids with polynucleotides, in contrast to the noneffects with free amino acids, are explained as a manifestation of molecular cooperativity.

Polymer-polymer interactions have been demonstrated with DNA and poly-L-lysine (Leng and Felsenfeld, 1966). Also, while significant polynucleotide-amino acid interactions have not been demonstrated, polyamino acid-mononucleotide complexes have been described, both with polyarginine by Woese (1968), and polylysine by Lacey and Pruitt (1970). Indeed, the fact that the polyamino acid-mononucleotide interaction is more easily demonstrated than the polynucleotide-amino acid interaction has been cited as a reason for believing that proteins were the first macromolecules in the origin of the contemporary genetic apparatus (Lacey and Mullins, 1972).

The principal inference, however, is that recognition can occur for either class of macromolecule, polyamino acid or polynucleotide, by the other. We need not accept the hypothesis that the original translation was in the nucleic acid → protein direction; it could have been reverse translation (Fox and Dose, 1972).

An explanation for intermacromolecular recognition leaves at least one fundamental question unanswered. That is the question of how the process first operated dynamically in the cell, which we recognize as itself possessing dynamic biochemical processes (Baldwin, 1957).

This question of dynamic synthesis was first studied by Dr Nakashima in our laboratory through the agency of preformed nucleoproteinoid microparticles, which were suspended in solutions of adenylic acid anhydrides of amino acids. For the adenylates the four homocodonic amino acids: glycine (GGG), lysine (AAA), phenylalanine (UUU), and proline (CCC) were employed. The microparticles were assembled from lysinerich proteinoid of a codonic type (Fox *et al.*, 1971) and each of the enzymically synthesized homopolyribonucleotides. Under an empirically determined set of conditions, in which the ratio of polynucleotide to proteinoid appears to be significant, the polynucleotide which has been most effective in incorporating amino acids from adenylates into the microparticles is the codonically related polymer. This incorporation has been discussed (Nakashima and Fox, 1972).

Instead of continuing those studies as described, we have shifted to using ATP instead of aminoacyl adenylates, for two reasons. One is that aminoacyl adenylates are troublesome to prepare, store, and to monitor in reactions. The other is that contemporary organisms use ATP as their source of energy, and of organic phosphate. We therefore turned our attention to the particle-governed polymerizations described in the beginning of this paper.

In future experiments, we will be interested again in identifying conditions for dynamic codonic and anticodonic interactions, this time with ATP and with examination of the supernatant, or the supernatant and the particles both.

The experiments reported here have described to some degree how a replicating primordial cell would evolve toward a much more fully contemporary cell, i.e. how it would utilize the energy from ATP to polymerize amino acids and mononucleotides. Earlier experiments indicate the intrinsic basis for interactions to constitute a con-

temporary genetic mechanism and a genetic code. Obviously, some gaps remain in demonstrating the orchestration of processes; other evolutionary innovations, such as the trapping of solar energy, remain largely to be explained by experiments.

In outline, however, experiments demonstrate how there could have occurred an evolution of hydrophobicity from hypohydrous environment to structured cell, from geothermal energy to cellular phosphate energy, from environmental protoinformation (mixtures of amino acids) through protoprotein to RNA-controlled cellular peptide-bond synthesis, and from a largely heterotrophic, replicating protocell to nucleic acid-governed, macromolecule-synthesizing contemporary cell (Figure 7).

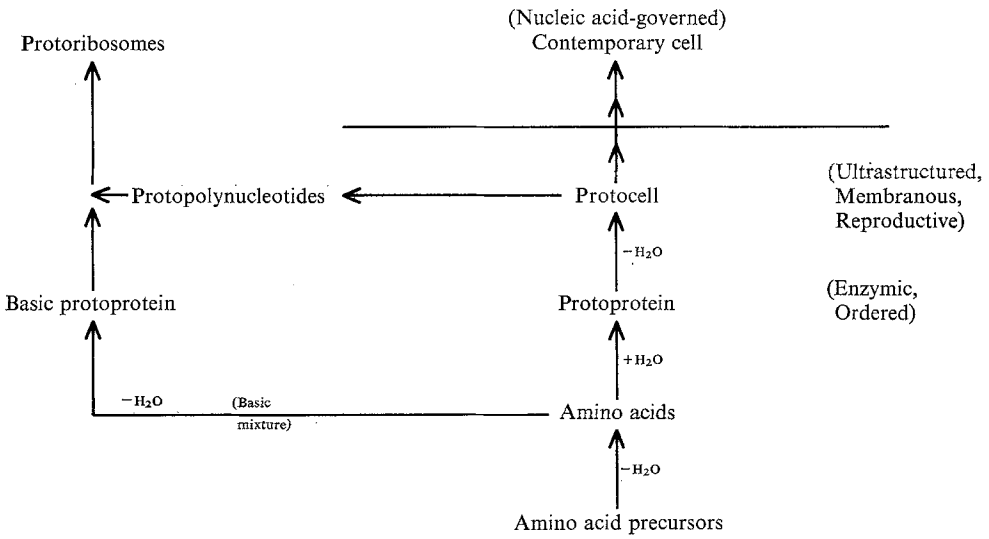


Fig. 7. Flowsheet of experimentally derived concepts, including emergence of protocell and contemporary cell.

The new experiments described are open to interpretation in two contexts. One is for the origin of cellular synthesis of biomacromolecules. What are the minimal requirements for synthesis of phosphodiester bonds or peptide bonds in a minimal cell? The results yield some definition that was previously lacking. The other context is the postulate that the proteinoid microsphere could evolve to a contemporary cell. This possibility has not been fully demonstrated, but evolvability of a model protocell has been demonstrated and the outlines of the total metamorphosis are clearer than they were. We are struck, however, as we were with the other properties of the proteinoid microsphere, by how many associated functions appear simultaneously.

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