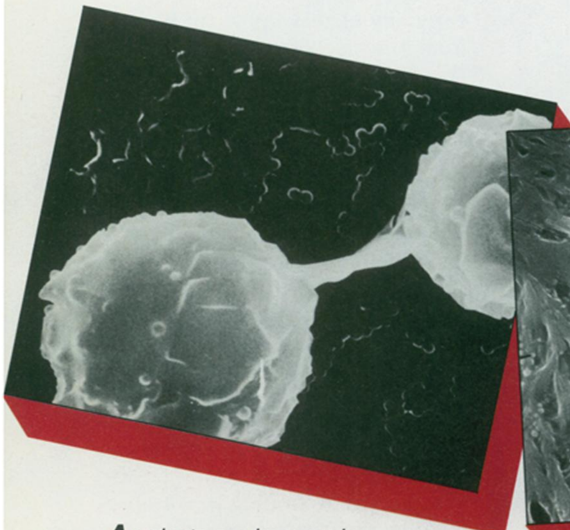
From Proteins to Protolife

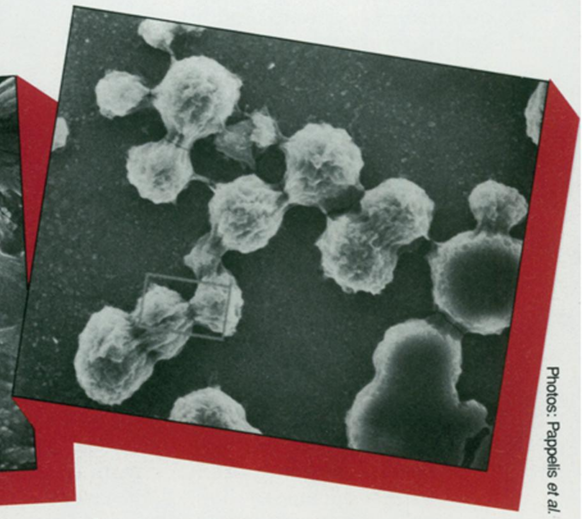
Was life's emergence random or guided by determined chemical steps?

By RICHARD LIPKIN



An electron microscopic image of two microspheres connected by a thermal protein tendril.

A cluster of microspheres in a gel-like thermal protein matrix.



A network of microspheres that has self-assembled and formed connections.

Photos: Pappelis *et al.*

The notion that life has evolved from lower, unicellular forms to higher, complex organisms is no longer considered a bizarre theory but rather, at least among the scientifically minded, a plausible premise.

Taken a step further, advances in biochemistry and genetics now show quite clearly that living organisms behave fundamentally as chemical machines, both driven by and limited by their molecular natures.

Therefore, given the premise that over millions of years nonliving molecules gave rise to living cells, one inevitably must ask: Did life emerge randomly? Was evolution accidental? Or were the chemical steps along the way constrained by the molecules involved and their inherent tendencies to aggregate in specific ways?

Could it be that the origin of life was not random at all, but instead a highly ordered, determined, chemical phenomenon?

More than 40 years have passed since Stanley L. Miller and the late Harold C. Urey, then biochemists at the University of Chicago, first showed that amino acids could form out of complex molecules under primordial conditions. In a famous 1953 experiment, Miller subjected the contents of a flask filled with ammonia, methane, hydrogen, and water to repeated cycles of heating, electrification, and cooling. The process produced a crimson-colored primordial soup, rich in amino acids.

In subsequent years, an interdisciplinary field of theoretical and experimental biochemistry, known as origin-of-life research, has itself evolved, driven primarily by biochemists eager to understand the fundamental, chemical mechanisms of prebiological molecules.

The field has orbited a central question: What molecular mechanisms and sequences of chemical steps prompted simple molecules to assemble themselves into living systems?

Among those early experimentalists was a protein chemist named Sidney W. Fox. In 1958, Fox and Kaoru Harada showed that under primordial conditions, amino acids could assemble themselves into simple proteins. At the time, this was startling news, since proteins serve as core structural components of living cells. In further work, Fox, now at the University of South Alabama in Mobile, and his colleagues observed that such proteins could fashion themselves into tiny cell-like objects called protein microspheres.

These microspheres look somewhat like empty cells, but without the internal machinery that runs a living cell. They even bear a striking resemblance to microfossils found in Precambrian rocks. They also demonstrate intriguing properties, joining together into networks and signaling each other electrically when stimulated by light.

Yet a host of questions has hovered for years around these protein spheres. Did they play a role in life's formation? Could they have led to the development, or served as precursors, of modern cells? Might thermal proteins—those forged from amino acids in a primordial broth by heat reactions—have provided enzymes to help build macromolecules or created a protected environment in which RNA, DNA, or their precursors could have formed?

Fox thinks so. He contends that thermal proteins formed from heated amino acids, assembled themselves into microspheres, and gave rise to protocells in the primordial environment. These protocells led to the subsequent evolution of nucleic acids and ultimately gave rise to self-sustaining cellular life.

The contention that proteins evolved before DNA or RNA is highly controversial. Indeed, Fox's "thermal protein first paradigm" runs counter to a central tenet of cellular biology—namely, that nucleic acids had to exist before any cell could arise that could properly be called living. In fact, most origin-of-life researchers point to the need for molecular mechanisms to store and replicate genetic information before evolution could commence. Scientists such as Stanley Miller and Francis Crick, a codiscoverer of DNA's structure, strongly emphasize RNA and its precursor molecules as necessary ingredients for prebiological systems.

Fox does not deny the importance or significance of DNA or RNA. Rather, he believes that the presence of thermal proteins may have sparked the chemical process that led to the evolution of these macromolecules necessary for true cellular life. Since the two types of molecules best suited for communicating biological information are nucleic acids and proteins, Fox asserts that either could have served as an original source. In essence, thermal proteins, he believes, may have been the active agents that triggered the chemical evolution of life.

The origins and bioactive properties of proteins have been fairly well defined. In addition, experiments by several groups have shown that thermal proteins can self-assemble and behave as enzymes, inhibitors, and precursors of proteins found in contemporary living cells. They have also demonstrated an ability to generate and retain molecular information.

What kind of molecular information? Structure.

Since a biological molecule's function hinges on its physical structure, its ability to perform a task rests on its size, shape, and chemical configuration. Biochemical information is thus stored and transmitted as molecular shape. Fox maintains that thermal protein microspheres could have provided a protected environment in which complex information-rich macromolecules bearing genetic information could have assembled themselves.

"Sidney's work is very important and interesting, especially the fact that amino acids can be heated to make microspheres of that nature," says Cyril Ponnamperuma, a biochemist at the University of Maryland at College Park. "This phenomenon is very significant for origin-of-life studies, especially given the ease with which it happens. Critics out there keep asking, 'What's the value of these little billiard balls?' But the fact that these spheres form so readily, in a variety of forms, is very intriguing."

Ponnamperuma adds that some crucial tests remain. "Fox has to show that the spheres demonstrate the properties of a membrane by transferring radioactive isotopes from outside to inside. It's possible that the microspheres are models of the first cells and should be taken seriously."

"This is a highly determined sequence of events that occurred on Earth, gave rise to life, and made evolution possible."
—Pappelis

Moreover, Ponnamperuma is not so concerned about which came first, proteins or nucleic acids. "To me, it's purely an academic question. Time's not important. Amino acids form, the bases form, nucleotides form. Proteins and nucleic acids may have come together. The great abundance of amino acids under prebiotic conditions is important to consider. Based on what we now know, amino acids themselves have unique ordering processes."

Among the former skeptics who now find Fox's theory plausible is Aristotel Pappelis, a biologist at Southern Illinois University at Carbondale. Pappelis supports the idea that thermal proteins might have formed microspheres that became precursors of modern cells. "These early cell-like units are protocells," he asserts. "They are the smallest units of protolife."

Thermal proteins make up the protocells' membranes, which share many characteristics of living cells' lipid membranes, Pappelis says. These rudimentary walls surround nascent protoplasm, itself an unorganized thicket of thermal proteins. These membranes remain permeable to small molecules but not to large ones. Outside the protocell, the remaining proteins sometimes form fibrous networks linking protocells together.

If these experimental findings do represent what occurred chemically eons ago, then the encapsulation of thermal proteins might in fact have served as the first step in evolution, Pappelis contends.

At a meeting in May at the A.N. Bach Institute of Biochemistry in Moscow, Pappelis explained that thermal proteins, if continually heated and cooled, alternately unravel and fold back on themselves, prompting the molecules to reform into new configurations that lead to microspheres.

"This is thermal proteins' unique feature," Pappelis says. "Microspheres can encapsulate thermal proteins and yet are also bathed by them. And the sphere remains porous, allowing molecules to go in and out quite easily."

"If the reactions within a sphere help make macromolecules, then we're talking about a cage in which thermal proteins could help build nucleic acids like RNA or DNA. Perhaps thermal proteins can also help synthesize true proteins. If they can, then this could lead toward the development of protocells."

"Through this type of mechanism," he observes, "cellular evolution could have arisen from microspheres."

Given this view of a gradual, stepwise, and determined emergence of life from nonliving molecules to self-assembling, replicating systems, Pappelis recently suggested creating a separate biological classification called protolife.

In this domain, quasi-living, cell-like entities would be the forebears of organisms that exist in three phylogenetic groups: bacteria, archaea, eucharya. Using this classification, biochemists could begin to categorize and organize the sequential molecular processes and structures that must have preceded cellular life.

"At first, simple microspheres that couldn't do very much metabolically must have existed, followed by more complicated ones capable of doing much more," Pappelis postulates. "Probably some thermal proteins contained amino acid sequences that gave rise to protein synthesis, to DNA, to RNA right there inside the microsphere cages. These spheres should be called metaprotocells. That's where the first inklings of evolution appear. If RNA or DNA synthesis could begin inside a microsphere, helped by the thermal protein's enzymatic activity, then that's a road to cellular evolution—especially since these are self-directing steps."

"This is a highly determined sequence of events that occurred on Earth, gave rise to life, and made evolution possible," Pappelis speculates. "Probably, the same thing has happened elsewhere" in the universe. □