Coevolution of membranes and channels: 
A possible step in the origin of life

Saint Clair Cemin and Lee Smolin*

* Center for Gravitational Physics and Geometry
  Department of Physics
  The Pennsylvania State University
  University Park, PA, USA 16802

September 22, 1997

ABSTRACT
We propose a scenario for the origin of life based on the coevolution of lipid bilayer vesicles and protein channels.

* smolin@phys.psu.edu
1 Introduction: Assumptions

We propose here an idea concerning the origin of life which we believe to be novel, but closely related to some previous ideas on the subject\[1, 3\]. The basic idea is that the first self-reproducing entities, autonomous agents in Kauffman’s sense\[4\] were lipid vesicles with simple protein channels. We will argue that there is a plausible sequence of events whereby such entities could have arisen from lipids and amino acids present in some primordial body of water, and co-evolved to become a primitive form of stable, self-reproducing entities.

We begin by listing the assumptions on which our proposal is based.

- The first self-reproducing entities arose in solution in lakes, oceans or pools of water on the early earth. They arose from interactions of small molecules formed by the passage of energy through the medium, by the Miller-Urey synthesis or some analogue\[5\]. The energy may have come from heat, lightning, reaction of redox couples or solar photons.

- Experiment has shown that the passage of energy through a medium of simple molecules such as $CO_2$, $NH_3$, $CH_4$ and $H_2O$ may result in the formation of amino acids\[5\]. Lipids and amphiphilic molecules are also observed to form under similar primitive conditions\[6, 2\]. Once formed they spontaneously form bilayer membranes whose minimal energy configurations are hollow vesicles\[6, 2\]. Such vesicles have even been seen to form spontaneously in material dissolved from meteorites\[7\].

- At the same time the evidence seems to indicate that nucleic acids do not form as readily or abundantly in such simple processes\[8\]. Although the hypothesis that the first living things were RNA molecules has been widely considered, there has yet to be discovered a convincing scenario whereby the ingredients for RNA could have formed in solution from prebiotic materials\[8\]. Nor is there yet a convincing scenario for the assembly of RNA or DNA spontaneously from its components, in the absence of the action of enzymes\[8\].

Nucleic acids involve a higher stage of chemical complexity\[2, 10\]. They also require special conditions present within cell membranes and

\[1\]There are interesting suggestions about the role of clay\[9\] in the origin of life, but these suffer from an important problem which a vesicle first scenario does not, which is how a network of chemical reactions that develops using clay as a kind of enzyme should transmute itself to one that has no trace of a role for clay or its constituents in it\[2\].
absent in sea water, such as the absence of calcium, which degrades them. Therefore it seems unlikely that nucleic acids evolved until after there were primitive vesicles with a mechanism for excluding calcium.

- It seems most probable that the first self-reproducing entities formed from the most common materials out of which they may be constituted. It therefore seems rational to ask if there is a possible scenario by which amino acids and lipids might have constituted the first self-reproducing entities. If so, given the evidence that they form more easily and copiously than nucleic acids in plausible prebiotic environment, it seems worthwhile to investigate the hypothesis that the first self-reproducing entities were composed solely of lipids and amino acids.

- It is known experimentally that polar lipids will spontaneously form vesicles formed from bilayers in solution \(\text{[6, 2]}\). These may spontaneously grow by the accumulation of lipid molecules and they have been observed to spontaneously divide \(\text{[6]}\). It thus seems most parsimonious to assume that the first self-reproducing entities involved these lipid bilayer vesicles \(\text{[2]}\). Given that they both form spontaneously in models of primordial environments and are in fact essential components of living cells it would be strange if they were not part of the first self-reproducing entities.

- At some early stage some primitive metabolism must spontaneously begin and it must be coupled to the early self-reproducing entities. It is natural to search for the origins of such processes in the phase separation accorded by the bilayer lipid vesicles. We will not discuss here the possible nature of the primitive metabolism, except to assume that it begins spontaneously within vesicles when they are illuminated by light. All that is needed is that light be converted to some form of potential energy by bonding or dissociating small molecules. The vesicle plays a role to contain the molecules involved, and prevent their dilution. Plausible scenarios for how this may have occurred, for example from the presence of chromophores dissolved in the interiors of the membrane vesicles, are described in \(\text{[2, 10]}\).

There is, however, a serious problem with the hypothesis that the first self-reproducing entities were composed solely of lipids and amino acids.

---

\(\text{[2]}\) The suggestion that the origin of life began with the spontaneous formation of lipid vesicles is of course very old. See, for example \(\text{[1, 2, 10]}\) and references contained therein.
self-reproducing entities involved only lipid vesicles coupled to some simple metabolism. This is the osmotic crisis\textsuperscript{[11]}. The problem arises because, as just mentioned, simple metabolic processes will be more likely to occur in the interior of lipid vesicles, as the concentration of their products will not be diluted by diffusion, because they cannot pass through the membrane. On the other hand, for exactly the same reason, any concentration difference in a molecule or ion incapable of diffusing through the membrane leads to a buildup of an osmotic pressure across the membrane. If not somehow vented, the pressure will rupture the membranes. Thus, it seems that there is a kind of paradox concerning the role of membranes in the origin of metabolism. Membranes may have played an essential role by concentrating the products of metabolism so that some autocatalytic cycles may establish themselves. At the same time, any sufficiently active metabolism results in the buildup of osmotic pressure which ruptures the membranes. We will refer to this as the osmotic paradox.

- Since amino acids also form spontaneously by passage of energy through plausible primordial media, it is natural to look for a role of proteins also in the first self-reproducing entities. Here, however, we meet a problem, which is that amino acids do not appear to spontaneously form themselves into proteins. The reasons for this are two-fold: First the binding of amino acids into proteins produces water, and this is not favored in an aqueous environment. Second, the rates at which amino acids at relatively low concentrations in solution meet each other are not very high.

There may be some spontaneous formation of short polypeptides through processes such as repeated dehydration. These may have occurred in puddles of water, repeatedly filled by rainwater and evaporated by sunlight. The problem is that the periodic evaporation poses a problem for the survival of any entities formed in the puddle. And if, on the other hand, the contents of such puddles were from time to time washed into a lake or ocean, there would be the immediate problem of the dillusion of the polypeptides.
2 Interactions of amino acids and lipid membranes

Given the assumptions we have just listed, it is natural to look for an origin of the first self-reproducing entities in some interaction of amino acids and lipid membranes. It seems quite plausible that both were found to significant concentration in some primitive aqueous environments. However, by themselves, each component has a problem that seems to block its alone constituting the first self-reproducing entity. In the case of the lipid membranes it is the osmotic paradox we described. In the case of the amino acids it is the fact that they do not spontaneously form into proteins.

Living things, by definitions, are entities that ensure their survival by solving whatever problems they are faced with. (One formalization of this statement is Kauffman’s characterization of an autonomous agent.) It is then natural if the origin of life involves the discovery of the solution to a small set of problems that block the synthesis or survival of some set of molecules. Given that both lipid membranes and amino acids each face such a problem, which prevents them from individually constituting the first self-reproducing entities, in spite of their being common in plausible primitive environments, we may ask if there are mechanisms by which they could solve each others problem. In this case the first self-reproducing entities might arise by coevolution of the lipid boundaries and proteins.

Once this question is stated some natural hypotheses suggest themselves. One particularly attractive set are the following.

2.1 How the membranes may solve the proteins’ problem

- **H1** In an aqueous medium containing lipid bilayer vesicles and amino acids, concentrations of amino acids will develop on the outer surfaces of lipid vesicles. Some amino acids have hydrophobic groups, while others have both hydrophobic and hydrophilic ends. The hydrophobic groups will be attracted to the lipid-water boundary. The hydrophobic amino acids may also bury themselves inside the lipid membranes. The result may be a steady buildup of amino acids on the outer surface of the vesicles.

- **H2** Once present on the surface of the vesicle, amino acids will react to form polypeptides. These polypeptides will be randomly formed from the species of amino acids that develop concentrations on the surfaces of the vesicles. The reason for this is that the lipid-water boundary may solve both problems that block the binding of amino acids into
proteins in the aqueous medium. First, amino acids and polypeptides are far more likely to encounter each other when they are restricted to move on a two dimensional surface than in solution. The finite size of the vesicles increases this probability further, for as long as the vesicle survives it can accumulate an increasing surface density of amino acids by collecting those it encounters in the solution.

Second, the membrane provides a medium for energy transfer in which chemical potential energy couples to phonons in the surface. This can assist the formation of bonds between amino acids, and so overcome the barrier necessary to exclude water.

If these hypotheses are correct than the surfaces of vesicles will accumulate a surface density of amino acids and short, random peptides, that increase linearly for the time the vesicle remains stable. As vesicles have been shown to be stable over periods of days[6] significant concentrations of polypeptides may accumulate on their surfaces.

Here we may note an observation of Morowitz[2], which is that short polypeptides bound to lipid membranes may be able to catalyze reactions that would require longer proteins in solution. The reason is first, that the membrane may provide structural support for the active areas of the enzyme that in solution is provided by the longer protein. Second, the membrane may, as already noted, play a role transferring energy, that in solution would require a longer protein.

It follows from this observation that autocatalytic networks of polypeptides as envisioned by Kauffman[12] may be more likely to develop attached to lipid-water boundaries than in solution.

Finally, we may observe that the formation of polypeptides on the surface of a membrane may provide a mechanism for breaking chirality. By itself, the membrane, or course, does not break the chiral symmetry. But if a polypeptide is to form such that all of its hydrophobic groups are buried in the membrane, while all of its hydrophilic groups stick out, and if the chain is to have bend uniformly to form a circle or spiral on the surface, then chains of amino acids which are dominantly of a single chirality may be preferred energetically.

2.2 What the polypeptides may do for the vesicles

Even if the existence of proteins bound to the surface or embedded within the membrane of the lipid vesicle leads to the catalysis of metabolic reac-
tions, there is still a problem which must be overcome, which is the osmotic paradox. How this is overcome is the subject of the next hypothesis.

- **H3** There is a small, but non-vanishing probability for a short random polypeptide made of random sequences of amino acids that bind to membranes to bury themselves in the membrane, and thus form channels through the membrane. When this occurs, the osmotic crisis is solved, for the channel opened up by the polypeptide can allow the ions or molecules that are the source of the osmotic pressure to escape. This lowers the osmotic pressure, and so ensures that the primitive metabolic processes that are the source of the pressure do not destroy the vesicle that contains them.

This hypothesis may be restated the following way: The first biologically active proteins were primitive membrane channels. This closes the circle, the lipid membranes catalyze the synthesis of short, random polypeptides from amino acids, while some of the polypeptides so produced will serve as channels to release the pressure due to primitive metabolic processes.

There are several arguments which we believe support this hypothesis beyond the need to resolve the osmotic paradox, if vesicles which encapsulate primitive metabolic processes are to be stable.

First of all, it is likely that an exact shape or sequence of amino acids is not needed to form a primitive channel in the membrane. Any polypeptide formed on the surface of a lipid vesicle as described above will be made of amino acids that are either hydrophobic, or contain both hydrophobic and hydrophilic groups. These naturally want to bury pieces of themselves in the membrane. All that is needed is for the minimal energy state of such a polypeptide in the surface to be a circle or spiral of sufficient length for a channel to be drilled. As shown by the properties of antibiotics such as gramicidin, on the order of 10 amino acids is sufficient for a polypeptide to be a channel former.

In contrast, a good deal more specificity is required for a polypeptide that catalyze a chemical reaction. For this reason it makes sense to hypothesize that protein channels formed prior to enzymes.

If this last hypothesis is true than we would expect vesicles on which peptides had assembled themselves into channels to be more stable, and hence last longer, than those that do not. As we have already noted, there is a natural mechanism for vesicles to reproduce themselves by accumulation of lipids followed at some point by spontaneous division. To have evolution
of the vesicles and protein channels there must be also a process of self-reproduction of the peptides that form the primitive channels. We may note that processes in which short peptides catalyze their own reproduction have recently been observed\[14\]. We need then the final hypothesis.

- \textbf{H4} \textit{There is a non-vanishing probability for an autocatalytic network of polypeptides to develop on the surface of a vesicle, one of whose reactions would lead to the reproduction of polypeptides which form membrane channels.} When this occurs, that autocatalytic network, together with the vesicle on which it grows, will have the ability of reproducing itself, when the vesicle divides.

If these hypotheses are true one would expect to find somewhere in biology short polypeptides with the following two properties: 1) when they come into contact with a lipid membrane they burrow into it and drill a channel through it and 2) they are synthesized through some process which is not the standard template process. The reason is that, given the hypothesis we have made here, there must have evolved primitive mechanisms of synthesis and control of proteins in the period between the one described here and the eventual takeover of control of the mechanisms of the cell by nucleic acids. As these were prior to the nucleic acids and the formation of the machinery of protein synthesis from amino acid templates, the original method of synthesis must have involved a non-standard series of reactions. Given the principle that increases of complexity in biology almost always come from adding structure and processes, while preserving the original system\[10\], we would expect some of these pre-nucleic acid proteins to be still active somewhere in biology and to be synthesized by some non-standard, non-template process. If protein channels were among the pre-nucleic acid proteins, some polypeptides able to drill holes in membranes may have survived which are still non-template synthesized.

Remarkably, at least one class of polypeptides that fulfills exactly these functions does exist, it is the gramicidins\[13\]. These are a class of low molecular weight channel formers that are formed from 15 monomers. Remarkably, they are made of both L and D amino acids, which provides further evidence for their antiquity.
3 Conclusions

Several key questions remain if these rough ideas are to provide a useful starting point for experimental and theoretical study.

1) Might there be a mechanism for the protein channels reproduce themselves, using only the lipid membranes and proteins? We know that short peptides on the order of 32 amino acids are able to spontaneously reproduce themselves from shorter elements\[14\]. It would be very interesting to discover a short polypeptide that both forms channels in lipid membranes and catalyzes its own synthesis out of shorter polypeptide. Alternatively, what is required is only the discovery of a short protein channel which is part of an autocatalytic set of polypeptides which may be bound to the surface of a lipid bilayer. We may note that 100% faithful reproduction is not at all required, it would be sufficient to have something like a quasispecies of polypeptides that formed autocatalytic sets that reproduce themselves with sufficient fidelity to be self-sustaining.

2) We have kept a part of the story in the background, due to our ignorance. This is the form of the primitive metabolism that we hypothesize gets started in the interiors of the lipid vesicles\[2, 10\], whose action sets up the osmotic crisis. Presumably to become self-sustaining, the system of primitive protein channels must become coupled to the primitive metabolism. There are natural ways for this to happen, as the products of the metabolism in fact pass through the protein channels.

This is in fact necessary as the channels must have some specificity; their effect must be not just to open holes in the membranes but to control the passage of specific ions and molecules through it. It may then be natural for channels to evolve that gain energy from the osmotic pressure of the passage of ions or molecules through them, and then contribute this energy to the autocatalytic cycles that lead to their own reproduction. It would be important to identify possible mechanisms for this coupling.

3) One way to test these ideas would be to study the interaction of gramicidins and other channel formers with possibly primitive membranes such as those that form spontaneously in experiments.

4) It would also be of interest to catalogue and study those biological polypeptides and proteins that are either not template synthesized or include amino acids of the wrong chirality. These are candidates for primitive proteins from the conjectured pre-nucleic acid era. On the other side, those who hypothesize an RNA first scenario for the origin of life must supply a reasonable explanation for the occurrence of biological polypeptides that are
5) A slightly modified form of this proposal would regard the vesicles and the chemical reactions they encapsulate as initially part of the environment and the membrane channels themselves as the first autonomous agents, or self-reproducing entities. This picture would be relevant if there were a short polypeptide that reproduced itself, following something like the mechanism of [14] using energy gained by the release of ions (produced by the chemical reactions in the vesicle) through a channel that it opened up in the vesicle’s membrane. In the event that the new protein channel was released into solution there might first evolve a population of channel openers in solution that reproduced themselves by fastening onto a primitive vesicle and drilling a channel in it in order to gain the energy needed to catalyze the linkage of smaller polypeptides into new copies of themselves.

The cell would then form when a population of such channel openers evolved that could control the energy released sufficient to extend the lifetime of the vesicle. This would open the way to a coevolution of the vesicles and channel openers.

ACKNOWLEDGEMENTS

We are grateful especially to Stuart Kauffman and Harold Morowitz for many conversations about the origin of life and this particular proposal. We thank them and David Deamer and Andy Ellington also for very helpful comments on a draft of this paper. We are also grateful to Federico Moran and Peter Schuster for lectures on the origin of life which were helpful in formulating this proposal. Conversations with Mark Goulain, Albert Libchaber and Marcelo Magnasco about gramicidin and the origin of life in general were most helpful, and these were made possible by the hospitality of Prof. Nick Khuri at Rockefeller University. This work was supported by a NASA grant to The Santa Fe Institute.

References

[1] H. J. Morowitz, B. Heinz and D. Deamer, The chemical logic of a minimum protocell in Origins of Life and the Evolution of the Biosphere, 18 (1988); H. Morowitz, Phase separation, charge separation and biogenesis, Biosystems 14 (1981) 41; Folsome and Morowitz, Prebiological membranes, synthesis and properties, Space, Life Science 1 (1969) 538.
[2] H. J. Morowitz, *The Beginnings of Cellular Life*, Yale University Press, 1992.

[3] D.W. Deamer *Sources and synthesis of prebiotic amphiphiles*, in *Self-Production of Supramolecular Structures*, G.R. Fleishaker, S. Colonna and P.L. Luisi, eds. (Kluwer, Dordrecht, 1994);

[4] S. Kauffman, *Investigations* Santa Fe Institute Working Paper. 96-08-072 (1996).

[5] S.L. Miller and L. E. Orgel, *The Origins of Life on the Earth*, (Prentice Hall, New Jersey, 1974; S.W. Fox and K. Dose, *Molecular Evolution and the Origin of Life* (Dekker, New York, 1977)

[6] P.L. Luisi *The chemical implementation of autopoiesis* in G.R. Fleishaker, S. Colonna and P.L. Luisi, eds. *op. cit.*; P. Walde, *Self-reproducing vesicles* in G.R. Fleishaker, S. Colonna and P.L. Luisi, eds. *op. cit.*; H. Yanagawa, *The origin and organization of possible protocellular structure units*, in *The origin and evolution of the cell*, ed. H. Hartman and K. Matsuno, (World Scientific, Singapore)

[7] D.W. Deamer, *Boundary structures are formed by organic components of the Murchison carbonaceous chondrite*, Nature (317) 1985 792.

[8] J. P. Ferris *The prebiotic synthesis and replication of RNA oligomers: the transition from prebiotic molecules to the RNA world* in G.R. Fleishaker, S. Colonna and P.L. Luisi, eds. *op. cit.*.

[9] A. G. Cairns-Smith, *Seven Clues to the Origin of Life* (Cambridge University Press, 1985).

[10] H. J. Morowitz, *A theory of biochemical organization, metabolic pathways and evolution* Santa Fe Institute Working Paper 96-04-014.

[11] F. Moran, lectures, El Escorial summer school on physics and biology, July 1997.

[12] S. Kauffman, *The origins of order* (Oxford University Press, New York, 1993)

[13] O.S. Anderson, D. B. Sawyer and R.E. Koepp II *Modulation of channel function by the host bilayer* in Biomembrane, Structure and Function-The State of the Art, eds Bruce P. Gaber and K.R.K. Eawaran, Adenine Press (1992).
[14] D.H.Lee, J.R. Granja, J.A. Martinez, K.Severin and M.R. Ghadiri, Nature, vol. 382 (1996) 525-528.