GE PRIZE-WINNING ESSAY

The Emergence of Cells During the Origin of Life

In simple protocells, formed by encapsulating RNA inside vesicles, interactions between the RNA and the membrane lead to the emergence of essential cellular behaviors.

Irene A. Chen

Modern living organisms are organized into cells. Fundamentally, a cell consists of a genome, which carries information, and a membrane, which separates the genome from the external environment. By segregating individual genomes from one another, cellular organization is thought to be critical to the evolution of replicating systems (*1*, *2*). Some of the oldest known rocks on Earth (~3.5 billion years old) contain biochemical signatures of life and also contain tantalizing suggestions of cellular fossils (*3*). But how did early self-replicating chemicals give rise to the "cell" as a unified entity? The combination of a genome and membrane does not constitute a unified cell unless interactions between the components result in mutual benefit. Was it a lucky accident that genomes and membranes began to cooperate with each other (e.g., evolution of an enzyme to synthesize membrane lipids)? Or are there simple physicochemical mechanisms that promote interactions between any genome and membrane, leading to the emergence of cellular behaviors? We explored such mechanisms experimentally, using model protocells.

A protocell could be constructed by encapsulating a self-replicating genome inside a chemically simple, self-replicating membrane (*1*). This minimalist, forward-engineering approach is akin to early evolution, which must have also used a minimal set of components. RNA is a particularly elegant genomic material, because it can act as both information carrier and enzyme [e.g., as an RNA polymerase (*4*)]. The discovery that the ribosome contains a catalytic ribozyme core lends considerable weight to the theory that an RNA world preceded the modern DNA-RNA-protein world (*5*–*7*). For the membrane, fatty acids are simple amphiphilic molecules that self-assemble into bilayer vesicles. These vesicles have interesting self-reproducing properties, including the ability to undergo multiple cycles of growth and division (*8*). Fatty acids have been synthesized under a variety of prebiotic conditions and have been found on meteorites (*9*–*11*). To validate this experimental model, we showed that the hammerhead ribozyme, which catalyzes a self-cleavage (or ligation) reaction, is active when encapsulated in vesicles composed of fatty acid (myristoleic acid) and its cognate glycerol monoester (*12*).

During the origin of life, what behavior would demonstrate the emergence of the cell as a new level of biological organization? A defining behavior of living systems is Darwinian evolution, which may act at any level, including that of the gene and the cell. Using model protocells, we observed a competition between vesicles encapsulating RNA and empty vesicles (*13*). Vesicles encapsulating high concentrations of RNA experienced substantial osmotic stress, driving the uptake of fatty acid from unstressed membranes. This resulted in the transfer of ~25% of the membrane from empty vesicles to vesicles containing RNA, relieving the membrane tension caused by the osmotic gradient. The growth of the osmotically stressed vesicles and the reduction of the unstressed vesicles were measured by the fluorescence resonance energy transfer (FRET) between fluorescent dyes incorporated into the membrane.

We suggest that a similar process took place during early evolution—vesicles encapsulating highly active genomic replicators would generate osmotic pressure, causing them to "steal" membrane from other vesicles containing less active sequences. Genomic fitness (i.e., replicative ability) would be translated into cellular fitness as the genome and membrane increased together, moving the evolutionary unit from the replicating molecule to the whole cell. As soon as replicators became encapsulated, a primitive form of competition could emerge between cells (see the figure). Remarkably, this process does not require a chance increase in complexity (e.g., addition of a new enzyme), but instead relies only on the physical properties of a semipermeable membrane encapsulating solute.

In a complementary experiment, we also demonstrated how membrane fitness (i.e., growth) might contribute to cellular fitness. Fatty acid vesicles can grow spontaneously by incorporation of a feedstock, such as fatty acid micelles (*14*). We found that membrane growth generated a transmembrane pH gradient, due to the faster flip-flop of protonated fatty acid molecules incorporated into the outer leaflet of the membrane (*15*). Acidification of the vesicle interior was measured by an encapsulated pH-sensitive fluorescent dye (pyranine). Thus, a protocell might capture a substantial fraction (~12%) of the energy released during membrane growth and store it in the form of a pH gradient. In modern biological systems, pH gradients are widely used for energy storage and transduction. For a protocell, this energy might even be directly useful for driving cellular processes, such as the

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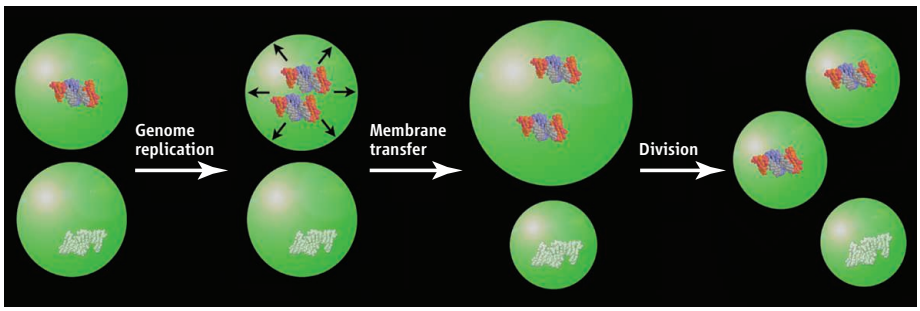

The emergence of cellular behavior. Competition emerges as protocells containing replicating genomes steal membrane from protocells containing inactive molecules.

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uptake of amines to aid RNA folding. Again, no additional enzymes need to be evolved for this basic form of energy capture and storage, which is only a consequence of the physical properties of the vesicles.

These results demonstrate that simple physicochemical properties of elementary protocells can give rise to essential cellular behaviors, including primitive forms of Darwinian competition and energy storage. Such preexisting, cooperative interactions between the membrane and encapsulated contents could greatly simplify the transition from replicating molecules to true cells. They also suggest intriguing possibilities for further investigation. For example, a corollary of vesicle competition is that a charged genetic polymer, such as nucleic acid, would be much more effective at driving membrane uptake than an electrically neutral polymer, because most of the osmotic pressure is due to counterions associated with the charged polymer. Could this influence the natural selection of the genetic material itself? Furthermore, competition for membrane molecules would favor stabilized membranes, suggesting a selective advantage for the evolution of cross-linked fatty acids (e.g., di- and triglycerides) and even the phospholipids of today. Greater membrane stability leads to decreased dynamics, however, and the evolutionary solutions to this problem (e.g., permeases, synthetic enzymes) could cause a "snowball" effect on the complexity of early life (*16*). Exploration of these minimal systems promises to lead to more exciting insights into the origins of biological complexity.

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2006 Grand Prize Winner

Irene A. Chen, the author of the prize-winning essay and a North American regional winner, was born in San Diego, California, to Taiwanese-American parents. She has had a fascination with science from a young age. As a high school senior, she won the Westinghouse Science Talent Search for research done under the direction of Carol MacLeod of the University of California, San Diego. She majored in chemistry at Harvard University, and as an undergraduate studied molecular recognition in the laboratory of Gregory Verdine. Dr. Chen stayed at Harvard to enter the M.D.-Ph.D. program. Under the mentorship of Jack Szostak, she investigated the biophysics of the origin of life—work that was recognized with a 2005 Harold M. Weintraub Graduate Student Award. She is currently finishing medical school at Harvard and plans to continue to study molecules and evolution.

Regional Winners

North America: Dianne Schwarz for her essay "Unraveling the Mysteries of Small RNAs." Dr. Schwarz received a B.S. degree from the State University of New York at Albany. She did undergraduate research in the laboratory of Caro-Beth Stewart, where she studied the function of short interspersed repeats in primate DNA. As a graduate student in Phillip D. Zamore's lab at the University of Massachusetts Medical School in Worcester, she characterized the RNA interference (RNAi) pathway in *Drosophila* and humans and investigated possible therapeutic applications of RNAi to diseases such as amyotrophic lateral sclerosis. Dr. Schwarz's thesis work was recognized with a 2005 Harold M. Weintraub Graduate Student Award. She is currently a Jane Coffin Childs postdoctoral fellow in the lab of Erin K. O'Shea at Harvard University, where she studies stress response in yeast.

Europe: Bernhard Loll for his essay "Photosystem II, a Bioenergetic Nanomachine." Dr. Loll was born in Ravensburg, Germany. He studied chemistry at Albert-Ludwigs-Universität in Freiburg, Germany, and received his diploma in 2000. During this time he worked in the group of Georg E. Schulz, and this stimulated his interest in biochemistry and protein crystallography. He continued to follow these interests by pursuing Ph.D. work in the group of Wolfram Saenger at Freie Universität Berlin. There, Dr. Loll elucidated the three-dimensional structure of photosystem II, in work done in cooperation with the group of Athina Zouni at Technische Universität Berlin. Dr. Loll defended his Ph.D. in February 2005 and is currently a postdoctoral scientist in the group of Anton Meinhart at the Max-Planck-Institut für Medizinische Forschung in Heidelberg.

All Other Countries: Ron Milo for his essay "Simple Building Blocks for Complex Networks." Dr. Milo grew up in Kfar Saba, Israel. As an undergraduate he studied physics and mathematics at the Hebrew University in Jerusalem. His Ph.D. research, conducted under the guidance of Uri Alon at the Weizmann Institute of Science in Rehovot, centered on analyzing complex biological networks with the use of network motifs. Dr. Milo continued as a postdoctoral fellow in the Alon group, where he measured the variability and memory of protein levels in human cells. His doctoral research was recognized with a Dimitris N. Chorafas Foundation Award in 2004 and the institute's John F. Kennedy Award in 2006. Dr. Milo is currently a fellow in the Department of Systems Biology at Harvard Medical School. In his spare time he enjoys investigating the beauty of nature in New England together with his wife and daughter.

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