SYSTEMS BIOLOGY, SYNTHETIC BIOLOGY AND THE ORIGIN OF LIFE David Deamer, Department of Biomolecular Engineering, University of California, Santa Cruz CA 95064. deamer@soe.ucsc.edu

Introduction: Over the past decade, systems biology and synthetic biology have emerged as two new sub-disciplines of biology. Although seemingly disparate, there are distinct regions of overlap that are worth exploring, particularly in terms of the origin of life. Systems biology is guided by our growing understanding that virtually all cellular processes are in the form of networks controlled by sensors, signals and effectors, properties that find analogies in the familiar control systems of modern technology. A few important life processes could even be characterized as digital, such as the genetic code, ribosome function, and the on-off firing of action potentials in neurons. These are being embedded in computational models that are expected to provide predictive insights into biological processes at the cellular level. In contrast, other important cellular functions occur through spontaneous self-assembly processes that are not under precise regulatory control, for instance, the insertion of lipids and certain proteins into membranes. Most other regulatory signals are essentially diffusion processes that exert their effects by summation over time and space. Examples include the diffusion of ions through channels that produces resting and action potentials of individual neurons and diffusion of neurotransmitters across synaptic clefts. The diffusion of growth factors also governs differentiation of cells into specific tissues within a developing embryo.

These regulatory functions are not in any sense digital, but the fact is that they work very well and life depends on them. The origin of life most clearly illustrates how a living system can emerge from a chaotic environment in the complete absence of anything we would call digital control. Instead it is better understood in terms of synthetic biology, currently defined in engineering terms with a primary goal of developing a "toolkit" that will allow manipulation of the genetic blueprint of living cells. However, the definitions of systems biology and synthetic biology can be usefully enlarged to encompass research on two fundamental problems facing biologists today: How did life begin? Can we fabricate a laboratory version of cellular life?

Discussion: The word *system* is derived from a Greek word having to do with being orderly, organized, connected. Today the word is widely applied to everything from political systems to solar systems, but here we will use it in a specific biological sense: *In living cells, systems are complex sets of molecular components that interact in order to carry out a specific function, and are regulated by a variety of control mechanisms.*

There are four general systems that are fundamental to all life today: a system of enzymes that catalyze and guide metabolic reactions, a second system of enzymes and membranes that produce energy for the cell, a third system of enzymes and ribosomes that synthesize proteins using the genetic information in nucleic acids, and a fourth system of enzymes that replicate the nucleic acids so that genetic information can be passed to the next generation. There are many other cellular systems, of course, such as those responsible for transport of nutrients across membranes, cell division, sensory response and motility, but the four outlined above are probably the most fundamental to the definition of life. (Some would add cell division and evolution to this list, but many kinds of cells are alive, for instance adult neurons, yet will never divide or evolve.)

The emergence of life on the early Earth involved the self-assembly of certain organic compounds into increasingly complex microscopic structures, so the term biocomplexity is useful in describing qualitatively how interacting molecular components gave rise to the first forms of life, followed by evolutionary processes leading to the contemporary biosphere. For instance, the sterile surface of the early Earth became more complex with the addition of organic compounds, either by synthesis or by delivery during accretion. In turn, the mixture of organic solutes became more complex over time as organic molecules underwent chemical evolution and began to assemble into molecular aggregates. Examples include the chemical synthesis of random polymers from suitable monomers such as amino acids, and the assembly of membranous vesicles from amphiphilic molecules. The nascent biosphere became much more complex at the origin of life nearly 4 billion years ago, when one or more of the self-assembled structures happened to have properties that allowed it to use energy to accumulate simpler molecules from the environment and assemble them into reproductions of the original structure. After life began, biocomplexity increased further as macromolecular structures became organized into systems within the cellular unit of life in order to catalyze metabolic pathways and to transmit information from one kind of molecule to another. The first forms of life that used primitive ribosomes for translation required a minimum of several hundred genes, and the simplest bacterial cells today use several thousand different catalytic and structural proteins to carry out their biological functions.

The origin of systems and the minimal cell

The origin of life can also be considered as the origin of molecular systems having certain specific properties. On the prebiotic Earth, countless numbers of natural experiments -- cellular compartments containing random mixes of polymers -- took part in a massive process of combinatorial chemistry. Life began when a rare few of the membranous compartments happened to contain a specific mix of macromolecules that allowed them to grow by energy-driven polymerization and then to replicate the macromolecules. Based on what we know about biological systems today, can we develop a hypothetical first living system? We can begin by enumerating and characterizing the parts of such a system, but it is also essential to define the control points. For instance, there must be feedback control between the polymerization reaction and the replication reaction, otherwise too much of one or the other macromolecule will be synthesized. A second feedback regulates growth of the membrane and growth of the polymers, and a third feedback regulates the synthesis of activated monomers. If these three processes are not regulated and synchronized with each other, one will inevitably grow too fast or lag behind, and the system will fall apart.

No one has yet attempted to develop an experimental system that incorporates all of the above components and controls, so we can only speculate on how control systems might have developed in early forms of life. There is one obvious point in the network that offers a place to start. Nothing can happen unless small nutrient molecules can get across the membrane boundary, so the rate at which this happens will clearly control the overall process of growth. The first control system in the origin of life may therefore have involved an interaction of internal macromolecules with the membrane boundary. The interaction represents the signal of the feedback loop, and the effector is the mechanism that governs permeability of the bilayer to small molecules. As internal macromolecules were synthesized during growth, the internal concentration of small monomeric molecules would be used up and growth would slow. But if the macromolecules disturbed the bilayer in such a way that permeability was increased, this would allow more small molecules to enter and support further growth, representing a positive feedback loop. The opposing negative feedback would occur if the disturbed bilayer could add amphiphilic molecules more rapidly, thus reducing the rate of inward transport by stabilizing the membrane.

Summary: The origin of life is best understood as occurring within a hierarchy of increasingly complex systems of molecules governed by chemical and physical laws. For life to begin, the core catalysts and information carriers were necessarily part of a system that included a container, a transporter and the ability to capture chemical energy from its surroundings. Beyond the simple fact that systems are organized into linear and branched networks, they also are controlled by regulatory processes involving feedback loops. Another property of biological systems is that specific protein components of the system undergo constant interactions. These can now be established by biochemical and genetic methods, resulting in a map of the interactions referred to as an interactome. A challenge for origins of life research is to understand the minimal interactome that will allow life to begin as a functional system of compartments and large molecules capable of catalysis and replication, together with feedback loops that regulate their functions. Finally, if we really do understand life in terms of systems biology, we should also be able to fabricate artificial versions of life in the laboratory. This is where systems biology meets synthetic biology, and the result will surely change the way that we view life on the Earth, not to mention the very real possibility that life has arisen on other planets by similar processes.