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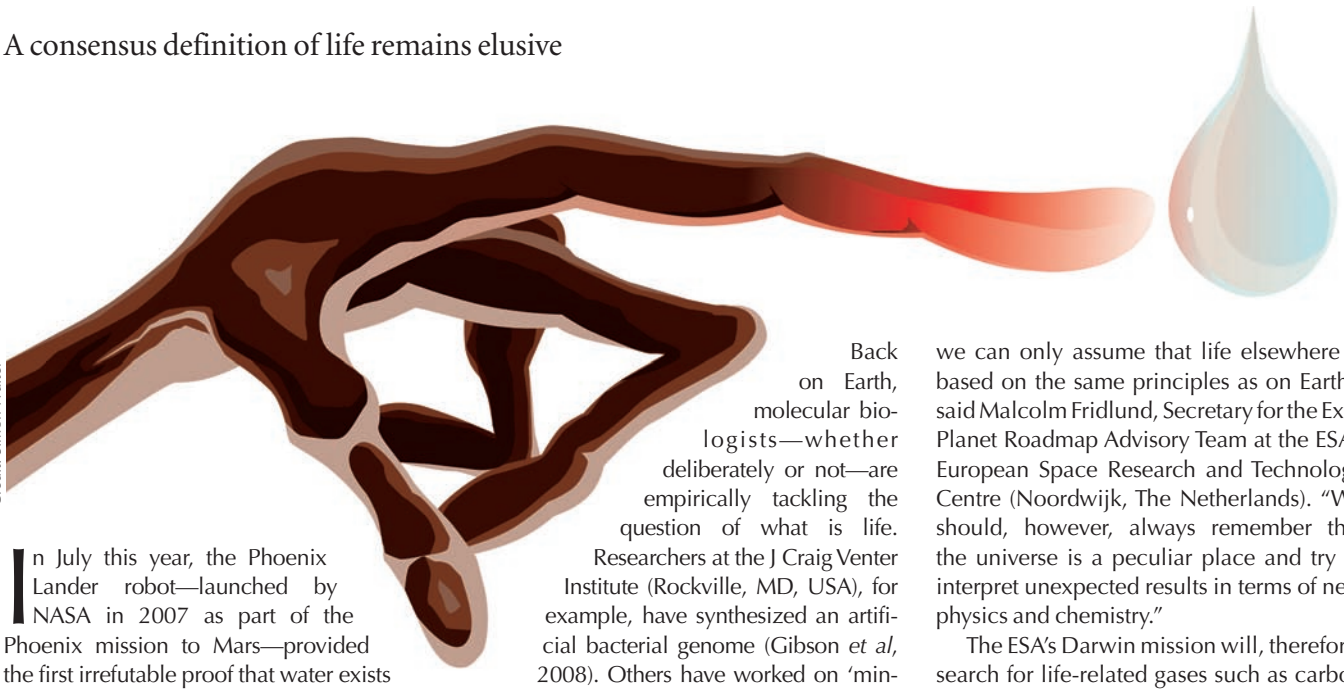
analysis

It's life, but just as we know it

A consensus definition of life remains elusive



Credit: Simon Walter

In July this year, the Phoenix Lander robot—launched by NASA in 2007 as part of the Phoenix mission to Mars—provided the first irrefutable proof that water exists on the Red Planet. "We've seen evidence for this water ice before in observations by the Mars Odyssey orbiter and in disappearing chunks observed by Phoenix [...], but this is the first time Martian water has been touched and tasted," commented lead scientist William Boynton from the University of Arizona, USA (NASA, 2008). The robot's discovery of water in a scooped-up soil sample increases the probability that there is, or was, life on Mars.

Meanwhile, the Darwin project, under development by the European Space Agency (ESA; Paris, France; www.esa.int/science/darwin), envisages a flotilla of four or five free-flying spacecraft to search for the chemical signatures of life in 25 to 50 planetary systems. Yet, in the vastness of space, to paraphrase the British astrophysicist Arthur Eddington (1822–1944), life might be not only stranger than we imagine, but also stranger than we can imagine. The limits of our current definitions of life raise the possibility that we would not be able to recognize an extra-terrestrial organism.

Back on Earth, molecular biologists—whether deliberately or not—are empirically tackling the question of what is life. Researchers at the J Craig Venter Institute (Rockville, MD, USA), for example, have synthesized an artificial bacterial genome (Gibson *et al*, 2008). Others have worked on 'minimal cells' with the aim of synthesizing a 'bioreactor' that contains the minimum of components necessary to be self-sustaining, reproduce and evolve. Some biologists regard these features as the hallmarks of life (Luisi, 2007). However, to decide who is first in the 'race to create life' requires a consensus definition of life itself. "A definition of the precise boundary between complex chemistry and life will be critical in deciding which group has succeeded in what might be regarded by the public as the world's first theology practical," commented Jamie Davies, Professor of Experimental Anatomy at the University of Edinburgh, UK.

For most biologists, defining life is a fascinating, fundamental, but largely academic question. It is, however, crucial for exobiologists looking for extra-terrestrial life on Mars, Jupiter's moon Europa, Saturn's moon Titan and on planets outside our solar system.

In their search for life, exobiologists base their working hypothesis on the only example to hand: life on Earth. "At the moment, we can only assume that life elsewhere is based on the same principles as on Earth," said Malcolm Fridlund, Secretary for the Exo-Planet Roadmap Advisory Team at the ESA's European Space Research and Technology Centre (Noordwijk, The Netherlands). "We should, however, always remember that the universe is a peculiar place and try to interpret unexpected results in terms of new physics and chemistry."

The ESA's Darwin mission will, therefore, search for life-related gases such as carbon dioxide, water, methane and ozone in the atmospheres of other planets. On Earth, the emergence of life altered the balance of atmospheric gases: living organisms produced all of the Earth's oxygen, which now accounts for one-fifth of the atmosphere. "If all life on Earth was extinguished, the oxygen in our atmosphere would disappear in less than 4 million years, which is a very short time as planets go—the Earth is 4.5 billion years old," Fridlund said. He added that organisms present in the early phases of life on Earth produced methane, which alters atmospheric composition compared with a planet devoid of life.

Although the Darwin project will use a pragmatic and specific definition of life, biologists, philosophers and science-fiction authors have devised numerous other definitions—none of which are entirely satisfactory. Some are based on basic physiological characteristics: a living organism must feed, grow, metabolize, respond to stimuli and reproduce. Others invoke metabolic definitions that define a living organism as having a distinct boundary—such as a membrane—which

facilitates interaction with the environment and transfers the raw materials needed to maintain its structure (Wharton, 2002). The minimal cell project, for example, defines cellular life as "the capability to display a concert of three main properties: self-maintenance (metabolism), reproduction and evolution. When these three properties are simultaneously present, we will have a full fledged cellular life" (Luisi, 2007). These concepts regard life as an emergent phenomenon arising from the interaction of non-living chemical components.

Cryptobiosis—hidden life, also known as anabiosis—and bacterial endospores challenge the physiological and metabolic elements of these definitions (Wharton, 2002). When the environment changes, certain organisms are able to undergo cryptobiosis—a state in which their metabolic activity either ceases reversibly or is barely discernible. Cryptobiosis allows the larvae of the African fly *Polypedilum vanderplanki* to survive desiccation for up to 17 years and temperatures ranging from –270 °C (liquid helium) to 106 °C (Watanabe *et al*, 2002). It also allows the cysts of the brine shrimp *Artemia* to survive desiccation, ultraviolet radiation, extremes of temperature (Wharton, 2002) and even toyshops, which sell the cysts as 'sea monkeys'. Organisms in a cryptobiotic state show characteristics that vary markedly from what we normally consider to be life, although they are certainly not dead. "[C]ryptobiosis is a unique state of biological organization", commented James Clegg, from the Bodega Marine Laboratory at the University of California (Davies, CA, USA), in an article in 2001 (Clegg, 2001). Bacterial endospores, which are the "hardiest known form of life on Earth" (Nicholson *et al*, 2000), are able to withstand almost any environment—perhaps even interplanetary space. Microbiologists isolated endospores of strict thermophiles from cold lake sediments and revived spores from samples some 100,000 years old (Nicholson *et al*, 2000).

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Another problem with the definitions of life is that these can expand beyond biology. The minimal cell project, for example, in common with most modern definitions of life, encompass the ability to undergo Darwinian evolution (Wharton, 2002). "To be considered alive, the organism needs to be able to undergo extensive genetic modification through natural selection," said Professor Paul Freemont from Imperial College London, UK, whose research interests encompass synthetic biology. But the virtual 'organisms' in computer simulations such as the *Game of Life* (www.bitstorm.org/gameoflife) and *Tierra* (http://life.ou.edu/tierra) also exhibit life-like characteristics, including growth, death and evolution—similar to robots and other artifical systems that attempt to mimic life (Guruprasad & Sekar, 2006). "At the moment, we have some problems differentiating these approaches from something biologists consider [to be] alive," Fridlund commented.

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Both the genetic code and all computer-programming languages are means of communicating large quantities of codified information, which adds another element to a comprehensive definition of life. Guenther Witzany, an Austrian philosopher, has developed a "theory of communicative nature" that, he claims, differentiates biotic and abiotic life. "Life is distinguished from non-living matter by language and communication," Witzany said. According to his theory, RNA and DNA use a 'molecular syntax' to make sense of the genetic code in a manner similar to language. This paragraph, for example, could contain the same words in a random order; it would be meaningless without syntactic and semantic rules. "The RNA/DNA language follows syntactic, semantic and pragmatic rules which are absent in [a] random-like mixture of nucleic acids," Witzany explained.

Yet, successful communication requires both a speaker using the rules and a listener who is aware of and can understand the syntax and semantics. For example, cells, tissues, organs and organisms communicate with each other to coordinate and organize their activities; in other words, they exchange signals that contain meaning. Noradrenaline binding to a β-adrenergic receptor in the bronchi communicates a signal that says 'dilate'. "If communication processes are deformed, destroyed or otherwise incorrectly mediated, both coordination and organisation of cellular life is damaged or disturbed, which can lead to disease," Witzany added. "Cellular life also interprets abiotic environmental circumstances—such as the availability of nutrients, temperature and so on—to generate appropriate behaviour."

Nonetheless, even definitions of life that include all the elements mentioned so far might still be incomplete. "One can make a very complex definition that covers life on the Earth, but what if we find life elsewhere and it is different? My opinion, shared by many, is that we don't have a clue of how life arose on Earth, even if there are some hypotheses," Fridlund said. "This underlies many of our problems defining life. Since we do not have a good minimum definition of life, it is hard or impossible to find out how life arose without observing the process. Nevertheless, I'm an optimist who believes the universe is understandable with some hard work and I think we will understand these issues one day."

Both synthetic biology and research on organisms that live in extreme conditions allow biologists to explore biological boundaries, which might help them to reach a consensual minimum definition of life, and understand how it arose and evolved. Life is certainly able to flourish in some remarkably hostile environments. *Thermus aquaticus,* for example, is metabolically optimal in the springs of Yellowstone National Park at temperatures between 75 °C and 80 °C. Another extremophile, *Deinococcus radiodurans*, has evolved a highly efficient biphasic system to repair radiation-induced DNA breaks (Misra *et al*, 2006) and, as Fridlund noted, "is remarkably resistant to gamma radiation and even lives in the cooling ponds of nuclear reactors."

In turn, synthetic biology allows for a detailed examination of the elements that define life, including the minimum set of genes required to create a living organism. Researchers at the J Craig Venter Institute, for example, have synthesized a 582,970-base-pair *Mycoplasma genitalium* genome containing all the genes of the wild-type bacteria, except one that they disrupted to block pathogenicity and allow for selection. 'Watermarks' at intergenic sites that tolerate transposon insertions identify the synthetic genome, which would otherwise be indistinguishable from the wild type (Gibson *et al*, 2008).

Yet, as Pier Luigi Luisi from the University of Roma in Italy remarked, even *M. genitalium* is relatively complex. "The question is whether such complexity is necessary for cellular life, or whether, instead, cellular life could, in principle, also be possible with a much lower number of molecular components", he said. After all, life probably did not start with cells that already contained thousands of genes (Luisi, 2007).

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To investigate further the minimum number of genes required for life, researchers are using minimal cell models: synthetic genomes that can be included in liposomes, which themselves show some life-like characteristics. Certain lipid vesicles are able to grow, divide and grow again, and can include polymerase enzymes to synthesize RNA from external substrates as well as functional translation apparatuses, including ribosomes (Deamer, 2005).

However, the requirement that an organism be subject to natural selection to be considered alive could prove to be a major hurdle for current attempts to create life. As Freemont commented: "Synthetic biologists could include the components that go into a cell and create an organism [that is] indistinguishable from one that evolved naturally and that can replicate […] We are beginning to get to grips with what makes the cell work. Including an element that undergoes natural selection is proving more intractable."

John Dupré, Professor of Philosophy of Science and Director of the Economic and Social Research Council (ESRC) Centre for Genomics in Society at the University of Exeter, UK, commented that synthetic biologists still approach the construction of a minimal organism with certain preconceptions. "All synthetic biology research assumes certain things about life and what it is, and any claims to have 'confirmed' certain intuitions—such as life is *not* a vital principle—aren't really adding empirical evidence for those intuitions. Anyone with the opposite intuition may simply refuse to admit that the objects in question are living," he said. "To the extent that synthetic biology is able to draw a clear line between life and non-life, this is only possible in relation to defining concepts brought to the research. For example, synthetic biologists may be able to determine the number of genes required for minimal function. Nevertheless, 'what counts as life' is unaffected by minimal genomics."

Partly because of these preconceptions, Dan Nicholson, a former molecular biologist now working at the ESRC Centre, commented that synthetic biology adds little to the understanding of life already gained from molecular biology and biochemistry. Nevertheless, he said, synthetic biology might allow us to go boldly into the realms of biological possibility where evolution has not gone before.

An engineered synthetic organism could, for example, express novel amino acids, proteins, nucleic acids or vesicular forms. A synthetic organism could use pyranosyl-RNA, which produces a stronger and more selective pairing system than the natural existent furanosyl-RNA (Bolli *et al*, 1997). Furthermore, the synthesis of proteins that do not exist in nature—so-called never-born proteins—could help scientists to understand why evolutionary pressures only selected certain structures.

As Luisi remarked, the ratio between the number of theoretically possible proteins containing 100 amino acids and the real number present in nature is close to the ratio between the space of the universe and the space of a single hydrogen atom, or the ratio between all the sand in the Sahara Desert and a single grain. Exploring never-born proteins could, therefore, allow synthetic biologists to determine whether particular physical, structural, catalytic, thermodynamic and other properties maximized the evolutionary fitness of natural proteins, or whether the current protein repertoire is predominately the result of chance (Luisi, 2007).

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"Synthetic biology also could conceivably help overcome the '$n=1$ problem'—namely, that we base biological theorising on terrestrial life only," Nicholson said. "In this way, synthetic biology could contribute to the development of a more general, broader understanding of what life is and how it might be defined."

No matter the uncertainties, researchers will continue their attempts to create life in the test tube—it is, after all, one of the greatest scientific challenges. Whether or not they succeed will depend partly on the definition of life that they use, though in any case, the research should yield numerous insights that are beneficial to biologists generally. "The process of creating a living system from chemical components will undoubtedly offer many rich insights into biology," Davies concluded. "However, the definition will, I fear, reflect politics more than biology. Any definition will, therefore, be subject to a lot of inter-lab political pressure. Definitions are also important for bioethical legislation and, as a result, reflect larger politics more than biology. In the final analysis, as with all science, deep understanding is more important than labelling with words."

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