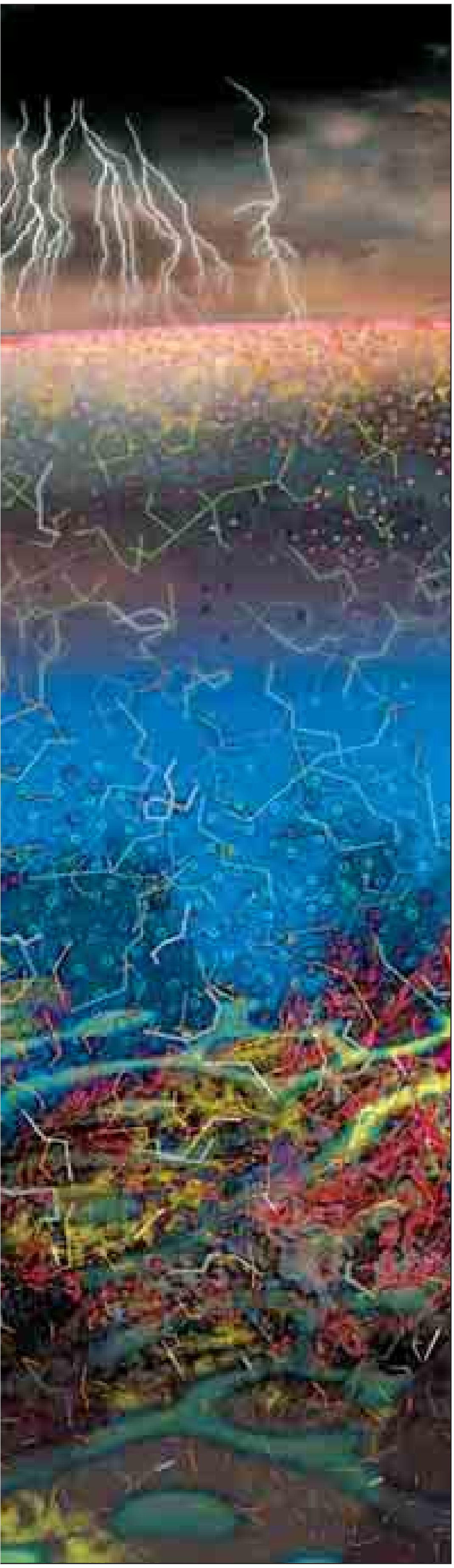


The chemical origins of Life on Earth

Soup, Spring, Vent or What?

We will never know precisely how life began several billion years ago; we can only collect evidence to design plausible scenarios. Jeremy Garwood discusses the current favourites.

Until the early 19th century, the prevailing view of spontaneous generation looked surprisingly similar to Aristotle's 2,000 year-old observation: aphids arose from the morning dew on plants, fleas from putrid matter and mice from dirty hay. Basically, it held that there was an ongoing spontaneous generation of certain life forms from non-living matter, a belief that was slowly overturned when microscopy revealed microorganisms and certain eloquent experiments demonstrated how covering food prevented unwelcome growth, e.g. in 1668 Francesco Redi proved that a mesh placed over meat sufficed to prevent fly maggots from appearing.

In 1861, Louis Pasteur's definitive demonstration showed that microorganisms do not spontaneously appear in sterile, nutrient-rich media seemed to settle the issue – life arose from life; all living cells came from pre-existing living cells.

Okay, well that's now, but what about long, long ago? In 1870, Thomas Huxley, celebrated as "Darwin's Bulldog" for his vigorous defence of the theory of evolution, held a talk comparing biogenesis and abiogenesis. He referred to abiogenesis as the spontaneous generation of life from non-living substances and explained that, while agreeing all known forms of life came from living ancestors, he thought that a process of abiogenesis must have once occurred on the primitive earth.

No doubt well aware of Huxley's ideas, Charles Darwin wrote upon the matter in a letter dated 1871, "It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and Oh! What a big "if"!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, etc., present that a protein compound was chemically formed ready to undergo still more complex changes. In the present day, such matter would be instantly devoured or absorbed, which could not have been the case before living creatures were formed."

The Primordial Soup

So, what kind of chemistry could have produced life? In 1924, Aleksandr Oparin, a young Soviet biochemist, argued that a primeval soup of organic molecules could have been created under the oxygen-less atmosphere of the early earth through the action of sunlight. The important point for him was that the early earth's atmosphere must have been reducing, since oxygen prevents the synthesis of some of life's key organic building blocks. Contemporary observations of Jupiter and Saturn had shown that they contained ammonia, methane and large amounts of hydrogen. These reducing atmospheres were regarded as captured remnants of the solar nebula and the atmosphere of the early Earth was assumed by analogy to have been similar. Once formed, he proposed that these organic molecules would combine to form droplets that could grow by fusion with other droplets and "reproduce" through fission to form "daughter" droplets.

The first major experimental evidence that conditions on a primitive earth could produce bioorganic molecules came in 1953 when Harold Urey and his young PhD student, Stanley Miller, tacked together some basic lab equipment to recreate earth's primordial environment in their Chicago laboratory.

Harold Urey, Nobel Prize winning discoverer of deuterium, proposed the characteristics for a primordial broth under Oparin's reducing atmosphere of methane, ammonia and hydrogen gas. He suggested that, under such conditions, it would be experimentally possible to produce organic molecules from water and methane using ultraviolet radiation or lightning. The experimental apparatus that Miller used had a glass flask at the bottom, containing an "ocean" of water, which was heated, forcing water vapour to circulate through the apparatus. The flask at the top contained an "atmosphere" consisting of methane, ammonia and hydrogen gases and the circulating water vapour. This gaseous mixture was exposed to a continuous electrical discharge ("lightning"), causing them to interact. Water-soluble products from these reactions then passed through a condenser and dissolved in the mock ocean.

Miller left his apparatus running for a week – after a day, the water in the flask "became noticeably pink", after a week, it was "deep red and turbid". It also contained a newly synthesised mixture of amino acids and amines!

But since 1953, there have been other ideas about the conditions existing when proto-life began. The Earth spun more rapidly on its axis – a day lasted only four or five hours. The Moon was much closer, causing strong, frequent tides and storms. Giant meteorites pelted the planet, vaporising the ocean surface when they struck water and throwing up dust clouds that blocked warmth and light from the Sun when they struck land.

In particular, it has been questioned whether the atmosphere would have had such a highly reducing composition. Although there was unlikely to have been appreciable amounts of molecular oxygen, the model since the 1970's has favoured a carbon-dioxide rich atmosphere, arguing that the reducing atmosphere of methane and ammonia would have been extremely vulnerable to destruction by UV light and that, under the lower gravity of the early earth, hydrogen gas would have rapidly escaped into outer space.

However, in 2005, Feng Tian *et al.* presented new calculations, claiming that the balance between slow hydrogen escape and volcanic outgassing could have maintained a hydrogen atmosphere of more than 30% (*Science* 308: 1014-17). Under these conditions, the production of organic molecules through UV photolysis could have been "10^{10} kg/year", more than enough to make a fertile organic ocean soup. Indeed, attacking rival models for abiogenesis, they proclaimed that it "would have been orders of magnitude greater than the rate of either the synthesis of organic compounds in hydrothermal systems or the exogenous delivery of organic compounds to early Earth".

Tian's provocative findings were swiftly condemned by advocates of alternate theories. David Catling from Bristol University, for example, said they neglected UV absorption of all gases other than H_2, and ignored the effect of the Earth's magnetic field upon the temperature and density of ions that would have promoted the nonthermal escape of neutral hydrogen gas.

But more support for the 'soup theory' also came from Bruce Fegley's renewed analysis of chondrites. These stony meteorites are believed to be the building blocks of rocky planets like the Earth, asteroids and satellites. Fegley's research indicates that the gases escaping from chondritic material subject to high temperature and pressure on the early earth would have been highly reducing hydrogen, methane and ammonia. Furthermore, this atmosphere might have been more robust than previously calculated, since photochemically produced hydrocarbon aerosols could have formed a haze layer in the upper atmosphere, protecting the lower atmosphere from photochemical destruction – something that has been observed on Titan, the largest of Saturn's moons.

Stanley Miller performing his famous 'origin of life'-experiment in 1953

Greater Complexity – a staggering concept

Before addressing alternative models to the 'soup theory', it helps to consider what is likely to come next. Even if all the chemical building materials of life could have been synthesised in a soup, how could they have self-assembled into multimolecular aggregates and, eventually, cell-like structures?

In comparison to the conversion of organic soup into Life's last universal common ancestor (LUCA), the concept of evolution from bacteria-like organisms to humans almost seems like child's play. LUCA must have already possessed tremendously complex, entirely self-sufficient, biochemical machinery with the capacity to very slowly make itself even more complex.

Is it any easier to work backwards from simple life forms to their components? Let's consider the most elementary cells currently known (that are not permanently dependent on host-metabolism): the bacterium, *Mycoplasma genitalium* has 482 protein-coding genes (compared to more than 2,000 in *E. coli*), of which 387 appear to be essential. In 2004, Rosario Gil from the University of Valencia estimated that the "core of a minimal bacterial gene set" was 206 genes. This vital minimum comprised the DNA replication machinery, rudimentary DNA repair, machinery for transcription and translation, post-translational protein processing and degradation, one gene for cell division, substrate transport, basic energy metabolism, limited pentose sugar, lipid and nucleotide biosynthesis – it assumes that many organic components, such as amino acids, fatty acids and the nitrogenous bases, could be recovered from the environment.

Either way, it looks like an incredible feat.

The soup theorists admit that once you have enough monomers formed in the ocean, they need to be concentrated, perhaps in tidal pools, before forming polymers. But going from monomers to polymers is not so simple – in addition to basic organic monomers, soup experiments continue to produce other compounds that prevent polymer formation. There is also the question of chirality – life is very specific about which organic stereoisomers it uses. Furthermore, the most crucial challenge is not simply to form polymers but to make polymers that can self-organise to form complex structures that interact to form a protocell. And how did the self-replication of polymers allow protocells to develop and reproduce?

"Genes first" versus "Metabolism first"

Many theories seek to explain more advanced steps in the abiogenesis of self-organising, self-replicating protocells, but a fundamental distinction lies in the timing of four billion year-old events. Did nucleic acids appear early on and play a key role (the 'genes first' school) or was it the generation of biochemical reaction pathways that provided the necessary conditions for the appearance of nucleic acids (the 'metabolism first' school)? And, of course, in such a debate, it is not surprising to see people cobbling together bits from both of these schools to create hybrid models of their own.

Metabolism first – the Iron-Sulphur World

As an alternative to cold soup, there are those who prefer hot starters. A German patent lawyer, Günter Wächtershäuser, left research shortly after completing his organic chemistry PhD in 1965. Nevertheless, from the 1970's onwards, this hobby chemist has been picking holes in the existing theory of a primordial broth, developing other theories of his own. Finding that "the soup theory is more of a myth than a theory, because it doesn't explain anything," his forthright opinions and unusual background have often clashed with the more traditional style of the soup theorists.

Wächtershäuser's main criticism of oceanic abiogenesis is the "entropy problem" – in the early Earth's vast oceans, the dilution of the organic compounds makes any chemical reaction between two molecules unlikely and a meaningful encounter improbable. Wächtershäuser said that the molecules needed some place to meet, a surface where they could interact, and pinpointed the surfaces of iron-sulphur minerals such as pyrite, that abound around underwater hydrothermal vents. Furthermore, the formation of pyrite could serve as a chemical power plant, providing chemical energy to react volcanic gases.

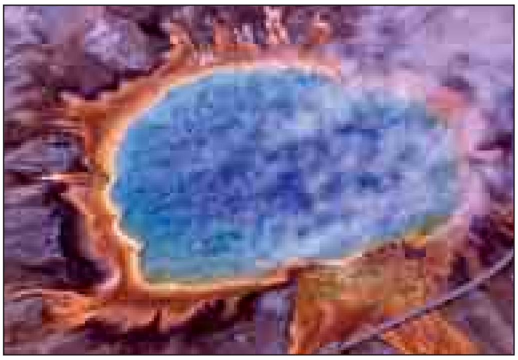
However, his ideas would probably never have entered the academic mainstream if Wächtershäuser hadn't become friends with Karl Popper, the distinguished philosopher of science, who encouraged him to write up his theories. In 1988, Popper communicated a paper to the *Proceedings of the National Academy of Science* (PNAS), Wächtershäuser's first scientific publication in 22 years. This paper was in fact a contribution to the RNA World "Genes-first" hypothesis, proposing an all-purine precursor to nucleic acids. It wasn't until 1990 that he published, "Evolution of the first metabolic cycles" (again communicated by Popper), composed of a mighty 5 Postulates and 11 Theorems, that proposed an autotrophic origin for the initial organic molecules (i.e. they could make their own chemical energy) rather than the heterotrophic soup model (in which the necessary energy came from the sun).

For Wächtershäuser, the central problem in this "metabolism-first" theory of an autotrophic origin was the very first process

Volcanic iron sulphur spring

of fixing carbon into organic molecules. He proposed an autocatalytic cycle that could be constructed by working backwards from the existing reductive citric acid cycle to arrive at the primitive core, notably replacing thioesters with thioacids and by assuming that the required reducing power was obtained from the oxidative formation of pyrite (FeS_2). This "strictly chemoautotrophic cycle is catalytic for pyrite formation and autocatalytic for its own multiplication."

However, at this point, it was just a theory. As Jeffrey Bada, Stanley Miller's most prominent ex-student, remarked, "Do, or did, the proposed chemical reactions actu-



Hot alkaline spring

ally take place in the real world? His theory is a bold step but there's probably nothing there, otherwise people would have found it already."

So, in addition to his work as a lawyer, Wächtershäuser had to find experimental proof. In 1994, he published a paper in *Nature* with Karl Stetter from Regensburg University, showing that pyrite formation could indeed be the driving force in the creation of amide bonds. Wächtershäuser then teamed up with Claudia Huber, a chemist at the Technical University in Munich. In a 1997 *Science* paper, the pair reproduced a key reaction, joining two carbon atoms to form activated acetic acid, a chemical at the core of many cellular metabolic pathways. A year later, they linked amino acids into short peptides, the precursors of proteins. In 2000, George Cody from Washington's Carnegie Institution reported creating pyruvate using a mineral catalyst under similar conditions – pyruvate with a three-carbon backbone is also a crucial component of living cells.

Armed with this experimental success, Wächtershäuser obtained research grants from the DFG, Germany's main research funding agency, so that he could continue to employ Claudia Huber (her university contract ended in 1998) and allow her to advance their lab research. In 2006, Huber and Wächtershäuser published their fourth *Science* paper, further exploring, "mechanisms for the build-up of bio-organic compounds by carbon fixation on catalytic transition metal precipitates". They described how the reaction of nickel hydroxide with hydrogen cyanide (HCN) generates nickel cyanide, which reacts with carbon monoxide (CO) to generate pairs of α-hydroxy and α-amino acids, e.g. glycolate/glycine, lactate/alanine, glycerate/serine, as well as pyruvic acid in significant quantities. They proclaimed that their results narrow the gap between biochemistry and volcanic geochemistry and open a new gateway for the exploration of a volcanic, hydrothermal origin of life.

For the principal soup theorists, including Stanley Miller, Bruce Fegley, Jeffrey Bada and Robert Hazen, this was too much. They vigorously disputed Huber's 2006 paper. They may have, "claimed to narrow the gap between biochemistry and volcanic geochemistry" but, "no plausible geological environment could maintain the cited conditions" – too much cyanide, too high a pressure of carbon monoxide. Furthermore, far from supporting a hot, volcanic origin (the pioneer metabolic theory) over a cold, oceanic origin (prebiotic soup), the results, "are easily accommodated within the framework of an updated prebiotic soup heterotrophic theory in which pyrite and other metal sulphites are an important source of electrons for reduction of organic compounds."

To which Wächtershäuser simply replied that his own cyanide chemistry was very similar to theirs, and that his use of higher CO pressures was, "simply to speed up the experiment". Indeed, he maintained, the fundamental difference between the two theories was that of slow versus fast: "the prebiotic soup theory is restricted to the testing of individual aspects of a long, protracted overall process. The pioneer organism, by contrast, is expected to be experimentally realisable in toto!"

Hydrothermal vents and the Alkaline Spring Hypothesis

However, even scientists who agree with the central theme of Wächtershäuser's iron-sulphur world say that he skates over the finer chemical details. "The energetics of Wächtershäuser's reactions are plain wrong," says Mike Russell, a geochemist at the University of Glasgow. "Pyrite, for instance, plays no role at all. I don't consider any of his stuff significant except his synthesis of activated acetic acid in 1997." Even then, acetic acid and pyruvate are still pretty simple compounds, how do you build more complex biomolecules?

Russell's basic equation for the production of a unit of proto-life looks more like this:

*{ 210 CO_2 + $H_2PO_4^{ñ}$ + Fe, Mn, Ni, Co, Zn^{2+} }**ocean** + { 427 H_2 + 10 NH_3 + HS^- } **hot spring** ⟶ { $C_{70}H_{129}O_{65}N_{10}P$ (Fe, Mn, Ni, Co, Zn) S }**proto-life** + { 70 $H_3C{\bullet}COOH$ + 219 H_2O }**waste***

His starting point considers the early earth's surface to have been unpleasant. "There was only one place of constancy and nourishment – a warm spring in the relative safety of the deep ocean floor. There, protected from destructive UV radiation, existed a place that never dried out, never got too hot or too cold, never became too acidic or too alkaline." A kind of chemical paradise.

In 1988, Russell and Allan Hall suggested that four billion years ago, abiogenesis occurred at warm, alkaline springs at the bottom of the acidic ocean. The germ of this alkaline-spring hypothesis came from a "chemical garden" that Russell made from a toy kit with his son. Long fingers of silica formed when hydrated crystals of a strong acid and weak base were immersed in an alkaline solution of sodium silicate. Russell's son then began breaking-up these fingers only to discover they were hollow. "Hollow!" Russell says he suddenly understood many puzzling mineral patterns he had observed in ancient rocks: they were iron sulphide columns, chimneys and bubbles that must once have been natural chemical gardens!

In this model, warm alkaline springs containing a reducing mix of hydrogen, sulphides and ammonia seeped out of hydrothermal mounds (hollow finger-like structures) into a cooler ocean that was acidic and oxidative, containing dissolved carbon dioxide, iron, phosphate and other vital trace elements. Here, "the gradients of temperature and pH would have provided energy and opportunity, and such a spring would have provided a constant source of chemical nourishment – a continuous regulated flow reactor." At the meeting of these waters, precipitates of iron-nickel sulphides form a membranous froth – inorganic, semi-permeable, gelatinous membranes that enclose hollow spaces in which the formation of nucleic acids and proteins could have been catalysed.

Support for Russell's theory came in 2000 when such structures were found at the 'Lost City' site, 800 metres under the Atlantic Ocean. Here, some of the spires are 60 meters high and the springs have a temperature around 90°C.

Wächtershäuser has continually modified his model, taking account of such findings. In his latest view of a volcanic iron-sulphur world, the "pioneer organism" arises from chemoautotrophic origins at sites of reducing volcanic exhalations. It has a composite structure combining an inorganic substructure and an organic superstructure. Within the surfaces of the inorganic substructure lie iron, cobalt, nickel and other transition metal centres that possess sulphido, carbonyl and other ligands. These are catalytically active and promote the growth of the organic superstructure through carbon fixation, a process driven by the reducing potential of the volcanic exhalations. The "pioneer metabolism" could reproduce by an autocatalytic feedback mechanism. Some organic products could serve as ligands for activating catalytic metal centres where they arose. "This unitary structure-function relationship of the pioneer organism later gave rise to the two major strands of evolution: cellularisation and the emergence of the genetic machinery."

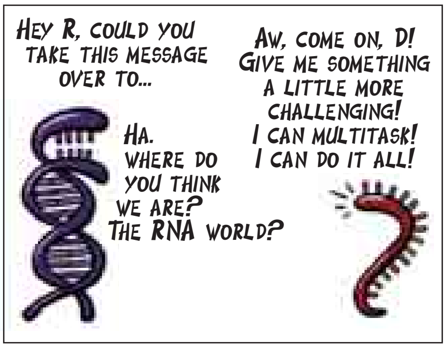
RNA World

First proposed in the 1960's by researchers such as Leslie Orgel, the RNA World hypothesis proposes that the earliest proto-life was based on ribonucleic acid (RNA), rather than DNA. It is argued that RNA has both the capacity to store information, like DNA, and to catalyse chemical reactions, like a protein-based enzyme. Such RNA-based catalysis and information storage could have been the first step in the evolution of cellular life, the RNA being encapsulated in early cell membranes formed spontaneously from proteinoids – protein-like molecules produced when heating amino acid solutions that can form microspheres. Alternatively, chemical reactions might have occurred inside clay substrates or, then again, on the surface of iron sulphide rocks.

Inevitably, RNA-based proto-life must have evolved towards DNA and protein. DNA, with its greater chemical stability took over the role of storing information, while protein can form many more catalytic structures from its greater variety of component amino acids. Indeed, the model suggests that RNA in modern cells is a remnant of the RNA World, especially ribosomal RNA that still catalyses protein production.

However, finding a mechanism for the prebiotic synthesis of nucleotides, let alone short RNA molecules, has been a major stumbling block. Ribonucleotides have three molecular components: a nucleobase (adenine, guanine, cytosine, or uracil), a ribose sugar and phosphate. Only adenine appears straightforward – this purine base is merely a pentamer of hydrogen cyanide and has already been found in simulations of the Miller-Urey soup experiment. But a 2009 paper from the group of John D. Sutherland at the University of Manchester now appears to have made a breakthrough (*Nature* 459(7244): 239-42). While the traditional search for prebiotic synthesis of pyrimidine ribonucleotides assumed that the ribose sugar and nucleobase components were formed separately and then combined, Powner *et al.* have now shown that a single 2-aminooxazole intermediate molecule could have contributed atoms to both the sugar and nucleobase, meaning they did not have to have formed separately.

Nevertheless, there has been more success at demonstrating that relatively short RNA molecules can catalyse their own continuing replication. Such replicase RNA, which functions as both code and catalyst, provides a template upon which copying can occur. In 1993, David Bartel and Jack Szostak showed that certain catalytic RNAs can join smaller RNA sequences together, creating the potential, in the right conditions, for self-replication. And, this February, Lincoln and Joyce from La Jolla's Scripps Institute reported the, "self-sustained replication of an RNA enzyme" (*Science* 323: 1229-32). They took a 76-nucleotide RNA enzyme



that can catalyse the RNA-templated joining of RNA and converted it, such that two of these RNA enzymes could now catalyse each other's synthesis from a total of four oligonucleotide substrates. In the absence of proteins or other biological materials, these cross-replicating RNA enzymes underwent self-sustained exponential amplification.

Synthesising life in the lab

Even if the key ingredients for life on earth came from extraterrestrial origins (on meteorites or in comet tails) as has been proposed by figures as distinguished as Francis Crick, we're still left with the same basic questions: how were they originally formed and what happened next? Several groups have decided that the final proof will be in the lab – they are going to generate proto-life itself!

Gunter Wächtershäuser's pioneer organism will, of course, be based on a self-organising chemical machine with the capacity to acquire self-replication. Meanwhile the Szostak lab at Harvard University aims to construct synthetic nucleic acid-based cellular life. There is also the hope that evolution of such an organism might be observable on the lab bench! And not to be left out of the big picture, Craig Venter and the Institute of Genomic Research have decided to work backwards from existing prokaryotic cells, removing more and more genes until they arrive at the point at which the minimum requirements for life have been reached – i.e. when one component will more or less make all the difference between life and a mixed bag of chemicals.

However, even if they all succeed, will we ever be able to say precisely how life began several billion years ago?