What magic ingredients transformed a seething broth of chemicals into the first living organisms? Phil Cohen describes some new twists in the search for the bare necessities of life

Let there be LIFE

AT 3·5 BILLION years old, fossilised bacteria are the earliest evidence of life on Earth, and yet these relics, with names like *Chroococcaceae* and *Oscillatoriaceae*, are identical to the sophisticated modern cyanobacteria that cover the globe from Antarctica to the Sahara. Evidence of any simpler incarnation fried in the intense heat of the young Earth before conditions were favourable for fossilising its remains.

In the absence of any rock-solid evidence, biologists have been free to speculate about the nature of the mysterious fledgling life form that came into existence some 4 billion years ago, and from which every plant, animal and microbe alive today eventually descended. All agree that early life, by definition, must have been capable of replicating and evolving. To do these things, most biologists have assumed that the ancestral life form needed a rudimentary instruction manual—a set of primitive genes—that was copied and passed from generation to generation.

In the past year or so, a majority view has emerged on which molecules first acquired these abilities and so sparked life on the planet Earth. Buoyed by some spectacular breakthroughs, most biologists are now convinced that life began when molecules called RNA took on the tasks that genes and proteins perform in today's sophisticated cells. In the once controversial "RNA world" theory, the chance production of largish RNA molecules was the crucial and committing step in the emergence of life itself. For many, this has become the only acceptable version of events.

But just when it looked safe to carve the RNA world theory in stone, its opponents are staging a spirited counterattack. Scientists in this second group don't agree on the details of their alternative visions, but they all make a claim that seems almost blasphemous in the era of molecular biology: far from being the first spark of life, they say, the RNA instruction manual was a mere evolutionary afterthought that helped fan its flames. What is more, they claim that the evidence proving their case will be in by the end of the decade.

A tricky problem

All modern life forms, be they germs, geraniums or Germans, have genes. The genes are made of DNA, which is made up of nucleotides; it is the sequence of these subunits that encodes the cell's instruction manual. The DNA is translated into RNA (also made up of nucleotides) which provides the blueprint for protein construction. The proteins, in turn, do all the metabolic grunt work, such as catalysing the chemical cycles that capture energy for the cell. They are also needed to translate DNA into RNA, and to make DNA copies to pass to the daughter cells. In other words, proteins, DNA and RNA are all essential for life as we know it.

For decades, this *ménage à trois* was the undoing of many a biologist trying to come up with believable scenarios for how life first appeared. Take away any one of the three and life grinds to a halt. But coming up with a plausible story for how DNA, RNA and proteins suddenly popped into existence simultaneously on a lifeless planet was just as tricky.

The first chink in this intellectual impasse appeared in the 1980s. Then Tom Cech at the University of Colorado and Sydney Altman at Yale University discovered that two naturally occurring RNA molecules sped up a reaction that snipped out regions of their own nucleotide sequence. RNA, it turned out, had some catalytic muscle of its own. The catalytic RNAs became known as ribozymes.

Theoreticians jumped on this discovery, envisaging a long ago world in which RNA ruled the planet. First, by virtue of its ability to act as a template for new RNA molecules, RNA was perfect for storing and passing on information. Second, by virtue of its ability to snap bonds between atoms, RNA was also a catalyst. Most crucial to the theory's credibility, the scientists proposed that RNA once catalysed the creation of fresh RNA molecules from their nucleotide building blocks.

Eventually, the free-wheeling RNA molecules would have acquired membranes and taken on additional catalytic tasks needed to run a primitive cell. But RNA's reign did not last. Under the pressure of natural selection, the proteins, which are better catalysts than RNA, and the DNA, which is less susceptible to chemical degradation, staged a cellular coup d'état, relegating RNA to its present role as a DNA-protein go-between.

Not surprisingly perhaps, those inclined to scepticism argued that it was too great a leap from showing that two RNA molecules partook in a bit of self mutilation in a test tube, to claiming that RNA was capable of running a cell single-handed and triggering the emergence of life on Earth.

Jack Szostak, a biochemist at Massachusetts General Hospital in Boston, set out to prove the sceptics wrong. He reasoned that the first RNA molecules on the prebiotic Earth were assembled randomly from nucleotides dissolved in rock pools. Among the trillions of short RNA molecules, there would have been one or two that could copy themselves —an ability that soon made them the dominant RNA on the planet.

To mimic this in the lab, Szostak and his colleagues took between 100 and 1000 trillion different RNA molecules, each around 200 nucleotides long, and tested their ability to perform one of the simplest catalytic tasks possible: cleaving another RNA molecule. They then carried out the lab equivalent of natural selection. They plucked out the few successful candidates and made millions of copies of them using protein enzymes. Then they mutated those RNAs, tested them again, replicated them again, and

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so on to "evolve" some ultra-effective new RNA-snipping ribozymes.

In the past few months, David Bartel, a biochemist at the Whitehead Institute for Biomedical Research near Boston and a former member of Szostak's team, has gone one better. He has evolved RNAs that are as efficient as some modern protein enzymes. The problem with most ribozymes is that they are as likely to snip an RNA molecule apart as stitch one together, which makes copying a molecule fifty nucleotides long (the minimum size necessary to catalyse a chemical reaction) a Sisyphean task. Bartel's new ribozymes, on the other hand, can stitch small pieces of RNA together without breaking larger molecules apart. What is more, his ribozymes use high-energy triphosphate bonds similar to ATP as their fuel, speeding the reaction up several million-fold.

"We've got ribozymes doing the right kind of chemistry to copy long molecules," says Szostak. "We haven't achieved self-replication from single nucleotides yet, but it is definitely within sight."

Electricity and hot air

Still, for the RNA world to have worked, it would have needed a supply of adenine, cytosine, guanine and uracil, the nucleic acid bases that, along with sugar and phosphate, make up nucleotides. Back in the 1950s, Stanley Miller, a 23-year-old doctoral student at the University of Chicago, announced that he had made amino acids, the pieces that click together to make proteins, with little more than a stuttering spark of electricity shot through hot air circulating in some glass tubing. The discovery was hailed as the first evidence that a lifeless planet could have spat out any of the raw materials needed for carbon-based life.

Miller's spark was a stand-in for primeval lightning, and the hot air, containing ammonia, hydrogen, water vapour and methane, was meant to mimic Earth's atmosphere 4 billion years ago. Besides creating amino acids, other researchers quickly demonstrated that the rich organic gook spewed out by Miller's decidedly non-biological combination also harboured chemical reactions that created huge amounts of adenine and guanine.

Cytosine and uracil, however, remained elusive. For this reason, and others, Miller's experiment did not convince everyone. Many atmospheric scientists argued that, unlike Miller's experimental setup, the incipient Earth was hydrogen-starved and entirely unsuitable for organic synthesis outside of a few havens, such as deep-ocean vents. This glitch led to the proposal of an alternative—to some fanciful—theory: that the organic building blocks came from outer space.

For much of his career, Jeffrey Bada, a geochemist at Scripps Institute of Oceanography, had argued that this was impossible. But a few months ago, Bada's own research forced him to change his mind. He found evidence that "mother lodes" of buckyballs have been delivered intact to Earth from outside the Solar System. Bada and his colleague Luann Becker made their find at Sudbury, Ontario, where a meteoroid the size of Mount Everest crashed 2 billion years ago. At first, Bada assumed that the buckyballs, football-shaped molecules made up of carbon atoms, had formed from vaporised carbon at the time of the impact. Then he discovered that they were loaded with helium, an element that has always been rare on Earth, but is abundant in interstellar space. What is more, the single impact site contained about 1 million tonnes of extraterrestrial buckyballs. If complex buckyballs could fall to Earth without being burnt up, so could complex organic molecules. "This blew our minds," says Bada. "We never expected it to be possible."

And while Bada's conversion was taking place, Miller, now at the University of California, San Diego, had not given

Joseph Jacobs

up on the idea that the primeval organic slime—wherever it came from—could have spawned the missing nucleic acid bases, cytosine and uracil. Last summer, 43 years after his original experiment, he and his student Michael Robertson discovered a way for the primordial pond to make them by the bucket-load. The secret ingredient was urea. Although urea is produced in Miller's original experimental setup, it never reaches a high enough concentration. But when he added more of the chemical, it reacted with cyanoacetaldehyde (another byproduct of the spark and hot air) churning out vast amounts of the two bases. Miller argues that urea would have reached high enough concentrations as shallow pools of water on the Earth's surface evaporated—the "drying lagoon hypothesis".

And in the last few months, another gap in the RNA world theory has been plugged. "The real question," says Jim Ferris, a chemist at Rensselaer Polytechnic Institute in Troy, New York, "is how did we get from a prebiotic concoction to [the first] long pieces of RNA? What was the bridge to the RNA world?"

In test-tube versions of the prebiotic world—as yet unblessed with protein enzymes or ribozymes—nucleotides link up, but only a few at a time. Once three or more have been connected, the RNA chain snaps—long before it has reached the magic length of fifty nucleotides needed to catalyse production of more RNAs.

In May, Ferris reported in *Nature* that he had found a means by which the first large chains could have been forged. When his team added montmorillonite, a positively charged clay that they think was plentiful on the young Earth, to a solution of negatively charged adenine nucleotides, it spawned RNA 10-15 nucleotides long. If these chains, which cling to the surface of the clay, were then repeatedly "fed" more nucleotides by washing them with the solution, they grew up to 55 nucleotides long.

The clay gets RNA off the hook of having to take on the tasks of information storage and catalysis in one fell swoop, says Ferris. It would catalyse RNA synthesis, stocking pools with a large range of RNA strands that, as Szostak and others have shown, would evolve a catalytic capacity of their own. In theory, an RNA catalyst would be born that could trigger its own replication from single nucleotides.

And with all the new evidence that is now available the apostles of the RNA world believe that their theory should be taken, if not as gospel, then as the nearest thing to truth that the science of the origins of life has to offer.

Not everyone agrees.

Power shortage

Evolutionary biologist Carl Wocse of the University of Illinois says the genetic evidence contradicts the RNA world theory. And if that weren't bad enough, he also argues that the RNA world scenario is fatally flawed because it fails to explain where the energy came from to fuel the production of the first RNA molecules, or the copies that would be

Bada: Mother lodes of buckyballs have been delivered intact to Earth from outside the Solar System needed to keep the whole thing going.

In test-tube RNA worlds, the elongating RNA molecules are fed artificially "activated" nucleotides, boosted with their own tri-phosphate bond to ensure that they come with an energy supply. In nature, such molecules only exist inside cells, and they have never been created in a Miller-type experiment. "The RNA world advocates view the soup as a battery, charged up and ready to go," Woese complains. On the primordial Earth, that energy had to come from somewhere, and it had to be coupled to production, or else it would quickly disappear into the ether.

In Woese's view, the critical step that ultimately spawned life was not a few stray RNA molecules, but the emergence of a biochemical machine that transformed energy into a form that was instantly available for the production of organic molecules.

The energy machine

Günter Wächtershäuser, an organic chemist at the University of Regensberg in Germany has suggested just such a machine. According to his picture, iron and sulphur in the primordial mix combined to form iron pyrites. Short, negatively charged organic molecules then stuck to its positively charged surface and "fed" off the energy liberated as more iron and sulphur reacted, creating longer organic molecules. The negatively charged surfaces of these molecules would attract more positively-charged pyrite, and the cycle would continue.

And by Wächtershäuser's reckoning, this energy-trapping cycle could easily have evolved into life forms that now exist—as chance ensured that one of the growing organic molecules was eventually of the right composition to catalyse its own synthesis. Ultimately, cycles of organic molecules would evolve that could trap their own energy—at which point they could do away with the inorganic energy cycle.

According to Woese, Wächtershäuser's theory and the RNA world theory are all testable, if only you know where to look for clues. The physical record of Earth's earliest life forms may have been erased, he says, but their "echoes, carried all the way through from pre-cellular times" remain encoded in the genes of modern organisms.

Six years ago, Woese, with Otto Kandler of the University of Munich and others, used those clues to transform our understanding of recent evolution. By using the mutation rates of genes as their guide, they pruned the tree of life, which traces how different species evolved, from five main sections to just three.

Woese says that a similar type of genetic analysis now shows that, contrary to the view of RNA world advocates, replication of RNA appears to have been a late development in evolution, and not its starting point. If RNA molecules had been responsible for the emergence of life, then the ancestral cell—which was supposedly descended from the initial RNA life forms, and the ancestor of all current life forms—would have had a sophisticated machinery for copying RNA. The genes encoding that machinery would have been subjected to selection pressures from the get-go, and so should be present in every modern organism in a relatively unaltered state. But, says Woese, when biologists look at these genes, species from the three branches of the tree of life have little in common. That shows, says Woese, that the machinery needed to copy RNA was a work in progress in the common ancestor cell, and that subsequent evolution on the three branches of the tree solved its inefficiencies in very different ways.

In short, RNA replication could not have been the trigger for the emergence of life. "Only its mere essence was there at the time of the common ancestor," Woese says.

And, he warns, "we're only beginning to unlock the secrets of the common ancestor." Comparisons of genes may soon reveal the identity of the first energy-producing metabolic cycle, he says. Assuming, for a moment, that the metabolic cycle was the initial life form, then when the first genes appeared they would have been co-opted into

Kauffman: **Life forms may exist that have no need of RNA or DNA or any other 'aperiodic solid'**

Woese: An energy-producing metabolic cycle, not RNA, triggered life on Earth

ratcheting up the efficiency of the metabolic cycle by producing enzymes to catalyse each step. These genes would then have been subjected to selection pressures for longer than any others, and should be present in all modern organisms in a similar state.

Until recently, an all-out search for this first metabolic cycle has been impossible, because only bits and bobs of DNA sequence were available from a few organisms. But genome projects are gathering momentum, spewing out complete sequences of organisms' every gene faster than the scientists can analyse them. This month, Woese and his colleagues plan to be the first to publish the sequence of an archaebacterium, *Methanococcus jannaschii*, a resident of boiling, deep-ocean vents. Woese predicts that 100 whole genome sequences will be in the databases by the end of the decade. Enough, perhaps, to finally track down the primordial energy cycle.

Woese and Wächtershäuser may be ruffling the feathers of RNA world enthusiasts by suggesting that an energy producing metabolic cycle, not RNA, triggered life on Earth. But Stuart Kauffman, a theoretical biologist at the Sante Fe Institute in New Mexico is leaving them speechless by suggesting that life forms may exist that have no need of RNA or DNA or any other "aperiodic solid". What is more, he says, the emergence of life wasn't some chance event, but something that was bound to happen under the conditions of the primitive Earth.

Out of chaos

Kauffman argues that the emergence of life on Earth is not the success story of a single type of molecule, such as RNA, slowly evolving to take on the catalytic burden of self-replication. In his view, the process was far more democratic. According to complexity theory, when a system reaches some critical level of complexity, whether it is made of stocks and shares or molecules, it naturally generates a degree of complex order. Likewise, he says, the mundane mix of nucleotides, lipids and amino acids that made up the primordial soup would in one magic instant have become an integrated system as the natural consequence of being part of a chaotic and complex mess.

Under such conditions, he says, self-replicating, "life-like" order is not a chance occurrence, it's a dead cert. In Kauffman' view, the modern *ménage à trois* of protein, RNA and DNA is not a conundrum, but a natural consequence of how life began.

He has demonstrated his theory using a computer model of the primordial stew. This shows that when a group of molecules—computer equivalents of simple organics with a few rudimentary catalytic skills—reach a critical level of diversity they spontaneously form an "autocatalytic set": a molecular cooperative that replicates as a group and evolves to create ever more complicated members. In other words, an autocatalytic set is a life form. What is more, says Kauffman, any sufficiently diverse mix—whether it is of carbon compounds or particles in an intergalactic dust cloud—will form autocatalytic sets, live, and evolve.

True, says Kauffman, RNA and DNA are part of all life today, but they arose as an accessory to an already flourishing ancestral autocatalytic set. Before genes existed, natural selection exerted its forces on the autocatalytic sets, ensuring that they were not biological dead-ends, but living systems capable of evolving to best suit their environment.

But many bench biologists scorn such ideas as cyberfantasy. "It's a pretty thought," says Gerald Joyce, who studies test-tube evolution at the Scripps Research Institute in San Diego. "But to be convinced, I need to see this autocatalytic gemish." And there's the rub. To prove Kauffman's theory you would need to analyse the contents of a pot in which percolated billions of different organic molecules, identify the autocatalytic entities and isolate them, and put them through their self-replicating cycles. Such an experiment stretches the bounds of what is technically feasible.

After years of trying to persuade the RNA world enthusiasts of the errors of their ways, however, Kauffman says he has gathered allies in biochemistry (he refuses to name names) who are willing to take on that task. He expects results in two to three years.

But in the short-term at least, most biologists say that the RNA world theory will prevail. Not unnaturally that worries those in opposition such as Woese, Kauffman, and Wächtershäuser.

"RNA chauvinism dominates the textbooks," says Gary Olsen, Woese's colleague at the University of Illinois. And that's a mistake, he warns, because the RNA world "as a theory it is only partly proven. The rest is speculative optimism." □

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