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MEET YOUR MAKER

What did the last common ancestor of all life look like? **Michael Le Page** delves into the primordial soup

IN 1859, when Charles Darwin published *On The Origin of Species*, he dedicated an entire chapter to the problem of missing "intermediate links" – transitional forms that bridged the evolutionary gaps between closely related species. If his theory was correct, the fossil record should be full of them. Where were they?

At the time it was a real problem, as few such fossils had been found. Then came the spectacular discovery, in 1861, of *Archaeopteryx*, with the wings and feathers of a bird and the teeth and tail of a dinosaur.

Since then we have discovered a multitude of intermediate links: fish that could crawl, lizards with mammal-like jaws, whales with legs, giraffes with short necks and many others. But there's one we are unlikely ever to find: the link between the earliest proto-life and life as we know it, also known as the last universal common ancestor, or LUCA.

LUCA lived around 4 billion years ago – a tiny, fragile life form that is the direct ancestor of every single living thing, from aardvarks to zebras. It wasn't the very first life: thousands, if not millions, of years of

evolutionary experimentation preceded it. But understanding LUCA would give us our best view yet of the origin of life.

We already know a surprising amount. Although any traces LUCA left in rocks were probably obliterated aeons ago, something far more revealing survives inside today's living cells: a biological operating system that is common to all life and must have been shared by LUCA too.

Many features of LUCA, though, have remained enigmatic, even paradoxical. But new work on a leading hypothesis for the origin of life might have solved many of the mysteries. It paints a detailed picture of where our earliest ancestor lived, how it lived and what it was like. Prepare to meet your maker.

Darwin himself was among the first scientists to speculate on how life originated: he envisaged a "warm little pond, with all sorts of ammonia and phosphoric salts, lights, heat, electricity, etc present". We will probably never know exactly how LUCA came to be, but we can make educated guesses by looking at some of the features of today's living systems.

These tell us much about what LUCA was like. We know it used DNA to store recipes for proteins, for instance. We even know what many of those recipes were, because many vital proteins found in all cells today must have come from LUCA. And from the nature of these proteins, it is clear that LUCA used an energy-rich molecule called ATP to fuel cellular processes, just as our cells do.

How did LUCA make its ATP? Anyone designing life from scratch would probably make ATP using chemical reactions inside the cell. But that's not how it is done. Instead, energy from food or sunlight is used to power a protein "pump" that shunts hydrogen ions – protons – out of the cell. This creates a difference in proton concentration, or a gradient, across the cell membrane. Protons then flow back into the cell through another protein embedded in the membrane, which uses the energy to produce ATP.

Think of a sink

To understand it in energy terms, think of a double kitchen sink. The small sink represents the inside of the cell and the large one the outside world. Start by filling the large sink with water, leaving the small one empty. The difference in water levels is a potential source of energy: drill a hole in the divider and water will flow into the small sink. The flow could be used to turn a tiny turbine – which is essentially what the ATP-making protein is, a turbine turned by protons and other positive ions (see diagram, page 32).

This process is so convoluted that when biochemist Peter Mitchell proposed it in 1961 it was dismissed as nonsense. But it has turned out to be common to all life, so most biologists think it must be how LUCA made ATP.

Exploiting a proton gradient requires a membrane that is impermeable to protons – they should only be able to flow in through the turbine. So it's assumed that LUCA had an impermeable membrane. But there is no evidence that this is the case. In fact, the nature of LUCA's membrane is an enigma.

To understand why, we have to backtrack to the 1970s, when it was thought that life could be divided into two great "empires". In one were animals, plants and fungi, and in the other the much simpler bacteria. Then microbiologist Carl Woese discovered that the bacterial empire actually contained two radically different types of life. A third "domain", now known as archaea, had been hiding in plain sight.

Archaea often look like bacteria, and are similar in many ways – as you would expect ›

> *"The building blocks of life would have formed spontaneously within the vents"*

given that both evolved from LUCA, probably quite soon after it existed. There are also some fundamental differences between them. One is their membranes.

Both bacteria and archaea have membranes made of water-repellent fatty molecules. Simple fatty molecules tend to flip around, making the membrane leaky, so both bacteria and archaea tacked on a water-loving phosphate group to stabilise the molecules and make their membranes impermeable. They took very different routes, though. Bacterial membranes are made of fatty acids bound to the phosphate group while archaeal membranes are made of isoprenes bonded to phosphate in a different way. This suggests that their membranes evolved independently.

This leads to something of a paradox: if LUCA already had an impermeable membrane for exploiting proton gradients, why would its descendents have independently evolved two different kinds of impermeable membrane?

Nick Lane of University College London, a biochemist and award-winning science writer, has come up with a startling answer that challenges many widely held ideas. Far from being impermeable, LUCA's membrane was leaky. In fact, he argues, it had to be leaky.

Lane starts from the assumption that life originated on the sea floor at places called alkaline hydrothermal vents. This was proposed in 1989 by Michael Russell of NASA. Its proponents, including Lane and William Martin of the University of Dusseldorf in Germany, have argued that it alone can explain why life uses proton gradients to generate ATP. Now, says Lane, it can also explain another of life's key features: the membranes of archaea and bacteria.

Distinct from the better known black smokers, alkaline hydrothermal vents are places where warm alkaline fluids, at temperatures of between 40°C and 90°C, well up through cracks in the sea floor. As the fluid hits the cold seawater, minerals precipitate out of solution, gradually forming rocky chimneys up to 60 metres tall, full of narrow channels and pores.


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Building blocks of life

Alkaline vents were present in primordial seas too. Within these ancient vents, Lane, Russell and Martin think, the building blocks of life would have formed spontaneously. The walls would have been rich in iron and sulphide, for example, which can catalyse complex organic reactions. What's more, temperature gradients within the pores should have created high concentrations of organic compounds and favoured the formation of large molecules, including lipids – fat molecules – and RNA.

So it would have been a perfect setting for the RNA world widely thought to have been the first step towards life. This may have been where self-replicating sets of RNA and other molecules first emerged and began to evolve into cell-like organisms with simple membranes. These proto-life forms needed energy – and it was provided, Martin and Lane argue, by the natural proton gradient at the interface between the proton-poor alkaline vent fluid and the proton-rich seawater. This is the ultimate origin of the proton gradients that power life today.

The stage was now set for the evolution, via a series of gradual steps, of the turbine protein that straddles the membrane and produces ATP. This was a crucial step in early evolution, though the cells could only survive at the interface between vent fluids and seawater, where there was a gradient to exploit. Only later did they evolve the ability to generate their own gradient using proton pumps.

It is a neat hypothesis but as critics have pointed out, there is a big catch. Early cells that had the ATP turbine protein but not proton pumps would only have been able to generate

Life powers up

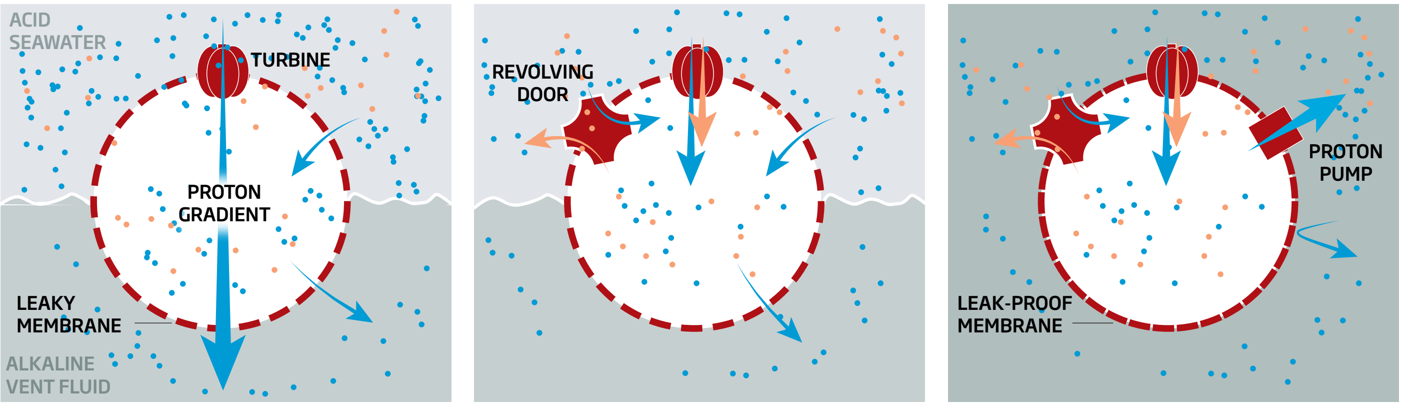
The earliest life on Earth could well have evolved at an undersea hydrothermal vent around 4 billion years ago. How did this cell get its energy, and how did it evolve to colonise the rest of the planet?

- PROTON
- SODIUM ION

The cell lives on the boundary between acid, proton-rich seawater, and alkaline vent fluid. It develops a protein that, like a turbine, extracts energy from the proton gradient (difference in concentration)

Later cells evolve a "revolving door" protein that effortlessly pumps sodium ions out while letting protons in. The sodium later re-enters through the turbine protein, generating even more energy

Eventually cells develop a dedicated proton pump and a leak-proof membrane. These cells can generate their own proton gradient across the membrane to obtain energy, and can leave the vent behind



Alkaline thermal vents on the sea floor: is this where life arose around 4 billion years ago?

a tiny amount of energy before the proton gradient collapsed. Without some means to shift protons out of the cell, the inside would quickly reach equilibrium with the outside.

To go back to the sink analogy, the water levels will rapidly equalise if water isn't being pumped out of the little sink. And so the hypothesis fails to explain how the universal ATP-making process arose. "It has worried me for some years," Lane says.

Think about it: you don't need a pump to restore the flow. All you have to do is to pull the plug out in the little sink. As it empties, the flow will resume. That is where the leaky membrane comes in.

Think of that primitive cell straddling the interface between seawater and vent fluid. One way to exploit the proton gradient is to have a leaky membrane. This would allow protons to flow continuously from seawater, through the cell, and back out into the vent fluid, without the gradient collapsing. As long as some of the incoming protons pass through ATP turbines, the cells have energy on tap.

Simple membranes made of fat molecules have just the required properties. In fact, fats spontaneously form cell-like structures that can grow and divide as more molecules are added. These properties mean they have long been of great interest to those exploring the origins of life. But the assumption was always that leak-proof membranes had to evolve before cells could exploit proton gradients. If Lane is correct, this is wrong.

Lane and Martin first sketched out the leaky membrane idea in 2012. Now Lane and his colleagues Andrew Pomiankowski and Victor Sojo have worked out a more detailed scenario and modelled it to see if it is energetically plausible. The results, just published in *PLoS Biology*, confirm that leaky cells – unlike impermeable ones – could extract enough energy from a natural proton gradient.

"Eventually, cells would have been able to break free altogether"

Yet in solving one problem, the leaky membrane creates another. At some point cells broke their umbilical connection to the vents. To do so, they had to evolve energy-consuming proton pumps to generate their own gradients. But if you've got a leaky membrane, there's nothing to be gained from pumping protons. They just leak back in again.

Free energy

The obvious solution is for the membrane to evolve to be less leaky. But this doesn't work either, because it would have stopped protons flowing through and shut off the energy supply. Catch-22.

Or is it? According to Lane there is a way out. Modern cells have a third kind of protein in their membranes. These act like revolving doors (see diagram, left), swapping one ion for another across the membrane – a sodium ion for a proton, say – and they don't use any energy.

A revolving door like this could solve the leaky membrane conundrum. If early cells evolved a revolving door that exchanged sodium ions for protons, the game suddenly changes. Sodium ions cannot pass through leaky lipid membranes nearly as easily as protons, so the exchanger converts the natural proton gradient into a sodium ion gradient across the membrane. Crucially, sodium ions can re-enter the cell through the ATP turbine protein (see diagram, below left). So a cell with an exchanger will get more flow through its turbines and thus generate more ATP – up to 60 per cent more, according to the model.

Once a cell has revolving doors, evolving a proton pump becomes advantageous even with a leaky membrane: the more protons it pumps out, the more it can swap for sodium ions and the more ATP it can generate. As pumping increases, making the membrane less leaky becomes an advantage as well. That means natural selection would have driven the joint evolution of better pumps and less leaky membranes.

Cells could then survive in weaker gradients at the peripheries of vents. Eventually they would have been able to break free altogether and generate their own proton gradient across a non-leaky membrane. And this is what they did – not once but twice, giving rise to the bacteria and the archaea.

Other early-life researchers, though, will take some convincing. "I think this scenario is highly unlikely," says Jack Szostak of the Howard Hughes Medical Institute in Boston, one of those who think that impermeable membranes evolved very early on. Cells with simple lipid membranes would leak not only ions but valuable metabolites, Szostak says.

How then did cells come to exploit proton gradients? That's not clear. Most researchers focus on very narrow features of early life, such as membranes or RNA. For now, only the alkaline vent scenario offers a broader picture, explaining not only where and how life came about, but why it has many of the peculiar features it does. "This paper is one of a series that deconstruct the organic soup hypothesis which has fooled us for almost 50 years now – that has always been blatantly anti-thermodynamic and hence basically out of the question," says Wolfgang Nitschke, a biochemist at the French national research agency in Marseille, who has studied the hydrothermal vent scenario.

This doesn't mean the vent scenario is right, but it does at least lead to predictions that can be put to the test.

For instance, can conditions like those in early alkaline vents really generate all the precursor molecules needed for life? Lane is trying to get funding to build a high-pressure reactor that would mimic the conditions at the deep-sea vents of 4 billion years ago.

In the meantime, consider this: we'll never know precisely what LUCA was like. But whatever it was, it lives on inside you. ■

Michael Le Page is a feature editor at *New Scientist*