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Scientists spent 12 years culturing a slow-growing, tentacled archaeon thought to be similar to the ancestor of complex cells.

THE MYSTERIOUS MICROBES AT THE ROOT OF COMPLEX LIFE

As scientists learn more about enigmatic archaea, they're finding clues about the origin of the complex cells that make up people, plants and more. **By Amber Dance**

Evolutionary biologist David Baum was thrilled to flick through a preprint in August 2019 and come face-to-face – well, face-to-cell – with a distant cousin. Baum, who works at the University of Wisconsin–Madison, was looking at an archaeon: a type of microorganism best known for living in extreme environments, such as deep-ocean vents and acid lakes. Archaea can look similar to bacteria, but have about as much in common with them as they do with a banana. The one in the bioRxiv preprint had tentacle-like projections, making the cells look like meatballs with some strands of spaghetti attached.

Baum had spent a lot of time imagining what humans' far-flung ancestors might look like, and this microbe was a perfect doppelgänger.

Archaea are more than just oddball lifeforms that thrive in unusual places – they turn out to be quite widespread. Moreover, they might hold the key to understanding how complex life evolved on Earth. Many scientists suspect that an ancient archaeon gave rise to the group of organisms known as eukaryotes, which include amoebae, mushrooms, plants and people – although it's also possible that both eukaryotes and archaea arose from some more distant common ancestor.

Eukaryotic cells are palatial structures with complex internal features, including a nucleus to house genetic material and separate compartments to generate energy and build proteins. A popular theory about their evolution suggests that they descended from an archaeon that, somewhere along the way, merged with another microbe.

But researchers have had trouble exploring this idea, in part because archaea can be hard to grow and study in the laboratory. The microbes have received so little attention that even the basics of their lifestyle – how they develop and divide, for example – remain largely mysterious.

Now, researchers could be closer than ever before to plausible evolutionary answers. Thanks to a surge in interest in these oft-overlooked microbes, and the ongoing invention of methods for tending to archaea in the lab, cell biologists are seeing them in more detail than was previously possible. Publications on this enigmatic group of microbes have nearly doubled over the past decade, and the nascent study of their biology is immensely exciting, says molecular microbiologist Iain Duggin at the University of Technology Sydney in Australia. "We can do some interesting fundamental experiments, and make some major first-step discoveries," he says. "We may be able to get a much clearer view of how the earliest eukaryotes evolved."

The images that wowed Baum, later published in *Nature*, offered such a view[1]. They were the result of 12 years' painstaking culture of an archaeon thought to be closely related to the one that spawned the eukaryotes. Microbiologists worldwide were thrilled by the portraits, but for Baum, they were a pet theory brought to life.

Five years earlier, he and his cousin, cell biologist Buzz Baum at the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB) in Cambridge, UK, had published a hypothesis about the origin of eukaryotes[2]. They predicted that the grandmother of them all might have sprouted protrusions, much like those on the archaeon in the paper. They reasoned that these protrusions came to surround nearby bacteria, which then transformed into a defining feature of eukaryotic cells: the lozenge-shaped energy-makers known as mitochondria.

As David Baum stared at the spaghetti-like strands, he recalls thinking, "Oh my goodness, we were right."

Fundamental mysteries

If a eukaryote is really a souped-up archaeon, then scientists must understand archaea to work out how the more-complex cells came to be. Whereas scientists studying eukaryotes and bacteria have been drilling down into processes such as cell division and growth for decades, the inner workings of archaea are still largely obscure. "Archaea, every time, do things differently," says Sonja Albers, a molecular microbiologist at the University of Freiburg in Germany. For example, related proteins might take on different jobs in different organisms. That makes archaea fascinating to study, says Duggin, but it's also important, because researchers can then compare across groups, looking for clues to the origin of the nucleus and other major innovations.

From the soils to the seas, one thing all cells have in common is that they split to make more of themselves. It happened in the common ancestor of all cell-based life on Earth, but the process started to look different as organisms adapted to their niches.

Researchers can explore evolution by looking at this divergence. Any mechanisms that all cellular life forms have in common point to biology inherited from the very earliest cells. By contrast, systems shared between only archaea and eukaryotes, or only bacteria and eukaryotes, hint at which parent provided the various ingredients of eukaryote biology. For example, the flexible membrane that separates eukaryotic cells from the outside environment resembles that in bacteria.

Duggin studies cell division in the archaeon *Haloferax volcanii*. It's a lover of salty conditions, such as those in the Dead Sea, and not of volcanoes, as the species moniker suggests. (It was named after microbiologist Benjamin Elazari Volcani.) For an extremophile, *H. volcanii* is pretty simple to grow in a salty broth, and its large, flat cells are easy to see dividing under the microscope.

Despite the enormous differences between bacteria, eukaryotes and archaea, the groups do share a couple of cell-division systems. In bacteria, a protein called FtsZ forms a ring at the future site of cell division. Duggin and his collaborators have observed the same in *H. volcanii*[3]. FtsZ, then, seems to have roots at the very base of the evolutionary tree.

Archaea have helped to surface other ancient proteins, too. One is SepF, a protein that Albers's group has found is essential to *H. volcanii* division[4]. Together with FtsZ, it could be part of a primordial "minimal system" for cell division, according to Nika Pende, an evolutionary biologist at the Pasteur Institute in Paris. Pende has analysed the distribution of the genes encoding FtsZ and SepF across a variety of microbes and traced them all the way back to the last universal common ancestor of all living cells[5].

Yet, at some point in evolution, some archaea assigned the cell-division job to a different set of proteins. This is where Buzz Baum's latest work comes in. His group has been studying the archaeon *Sulfolobus acidocaldarius*. In this case, the name fits: it loves acid and heat. Lab members wear gardening gloves to protect themselves from the acidic liquid it lives in, and built a special chamber so they could watch it divide under the microscope without cool spots or evaporation.

> **"Several dozen models that were tested have died along the way."**

Baum's team saw a completely different group of proteins managing the division ring. In eukaryotes, where they were first discovered, these proteins aren't just involved with division. They have a much broader role, pinching membranes apart all over the cell to create membrane-wrapped packages called vesicles, and other small containers. The proteins are known as ESCRTs (endosomal sorting complexes required for transport). In *S. acidocaldarius*, the team saw archaeal proteins related to these all-purpose pinchers managing the division ring[6], suggesting that early versions of ESCRTs evolved in the archaeal ancestor of eukaryotes.

FtsZ, meanwhile, evolved into eukaryotic tubulin, which gives structure to our cells. These discoveries suggest that the archaeal ancestor of eukaryotes probably had a kit for shaping and dividing cells that natural selection then adapted to the needs of the more complex descendant cells.

Glimpsing grandmother

But what kind of cell was that ancestor archaeon? And how did it meet, and merge with, its bacterial partners?

Biologist Lynn Margulis was the first to propose, in 1967, that eukaryotes arose when one cell swallowed others[7]. Most researchers agree that some engulfment went on, but they have different ideas about when that happened, and how the internal compartments in eukaryotes came about. "Several dozen models that were tested have died along the way because they're no longer plausible," says Sven Gould, an evolutionary cell biologist at Heinrich Heine University in Düsseldorf, Germany. Other theories might rise or fall as cell biologists add to their understanding of archaea.

Many models assume that the cells that eventually became eukaryotic were already quite complex, with flexible membranes and internal compartments, before they ever met the bacterium that was to become the mitochondrion. These theories require cells to have developed a way of gobbling up external material, known as phagocytosis, so they could snap up the passing bacterium in a fateful bite (see 'Two ways to make complex cells'). By contrast, Gould and others think that mitochondria were acquired early on, and that they then helped to fuel a larger, more complex cell.

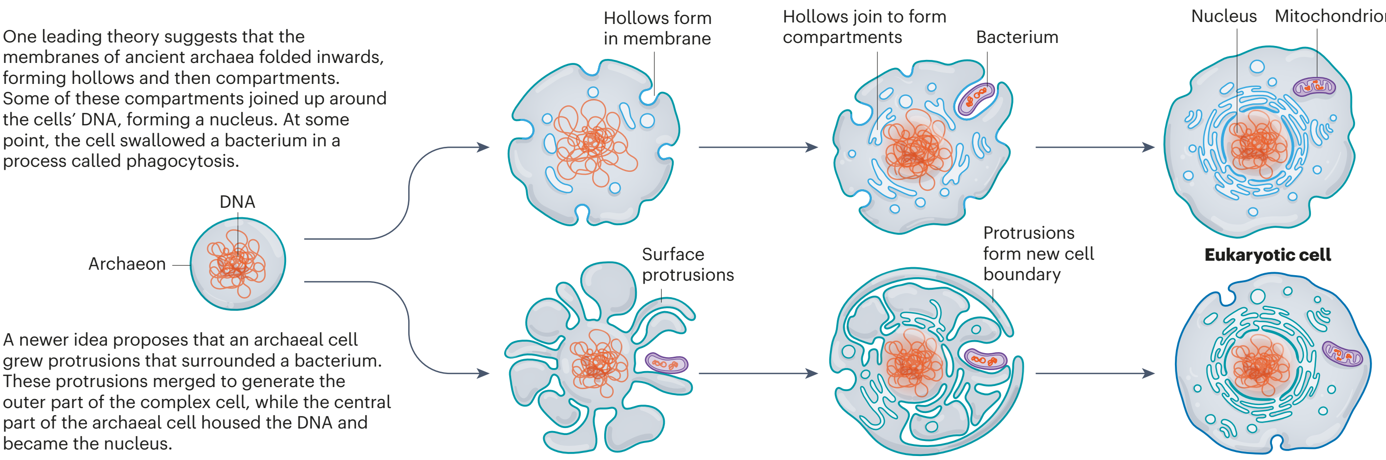
The Baums' model is one of few to explain how mitochondria could arise without phagocytosis. David Baum first came up with the idea as an undergraduate at the University of Oxford, UK, in 1984. His process starts with archaea and bacteria hanging out, sharing resources. The archaeon might start to stretch and bulge its exterior membranes to boost the surface area for nutrient exchange. With time, those bulges might spread and grow around the bacteria until the bacteria were, more or less, inside the archaeon. At the same time, the archaeon's original exterior membrane, now dwarfed by the long tentacles surrounding it, would evolve into the boundary of the new nucleus, while the cell's new exterior membrane would form when some particularly long tentacles grew right around the edge, greatly enlarging the cell compared to its archaeal precursor. This process differs from phagocytosis, in that it starts with a community of organisms and takes place over long timescales, rather than in a single bite.

David Baum's tutor told him the idea was creative, but lacking in evidence. He set it aside. But he'd already shared his enthusiasm for life science with his cousin Buzz, a child then, at regular family dinners in Oxford. "That's partly why I went into biology," recalls Buzz.

In 2013, David decided to write up his theory. He sent a note to Buzz, by now running his own lab, who helped develop the theory further. The duo defined several aspects of biology that support their idea, such as the fact that archaea and bacteria have been found living side by side and trading nutrients. The

TWO WAYS TO MAKE COMPLEX CELLS

Many researchers think that the cells of eukaryotes — organisms whose cells have complex internal structures — evolved when a bacterium merged with a type of microbe known as an archaeon. Over time, the bacteria became mitochondria, the energy-producing modules inside eukaryotic cells. But how did the union take place?



NIK SPENCER/*NATURE*; SOURCE: B. BAUM & D. A. BAUM *BMC BIOL.* **18**, 72 (2020).

Baums struggled to publish their proposal, but it finally found a home at *BMC Biology*[2] in 2014.

The idea received an enthusiastic response, Buzz recalls, especially from cell biologists. But back in 2014, David still thought they had just a 50–50 chance of being right.

And then, five years later, the spaghetti-and-meatball images appeared. Both Baums were thrilled.

The species was the first to be cultured from a group called the Asgard archaea. These organisms, described in 2015, have genes encoding proteins that many scientists consider remarkably similar to those of eukaryotes[8]. Researchers quickly came to suspect that the archaeal ancestor of eukaryotes was something akin to an Asgard archaeon. By pointing to a potential grandmother, the discovery supported the Baums' hypothesis.

The Asgard representative – which doesn't yet have a finalized name, and is currently known as *Candidatus* 'Prometheoarchaeum syntrophicum' – grew in a bioreactor alongside either of a pair of microbial hangers-on with which it shared nutrients. Notably, it lacked any complex internal membranes or signs that it could ever hope to phagocytose those associates. It had three systems that could be associated with cell division: proteins that are equivalent to FtsZ; ESCRTs; and the muscle-contraction protein actin, which also contributes to division in eukaryotes. The culturers haven't yet worked out which it uses to split itself, says team member Masaru Nobu, a microbiologist at the National Institute of Advanced Industrial Science and Technology in Tokyo.

The big surprise came when the cells stopped dividing and sprouted tentacles. It's possible, the Baums suggest, that these might amplify nutrient exchange with the microbes that the archaeon was co-cultured with, as their model predicted for the grandmother cell.

On the basis of their observations, Nobu and his colleagues developed a theory about how eukaryotes evolved that shares much with the Baums' idea. It involves one microbe extending filaments that eventually engulf its partner[1]. "I like our hypothesis because it allows for these complexities that are unique to eukaryotes" – nuclei and mitochondria – "happening at the same time", says Nobu.

Culturing confidence

The pictures of the Asgard archaeon really helped to shore up the Baums' theory. "It's very exciting that they form these protrusions," says evolutionary microbiologist and Asgard co-discoverer Anja Spang at the NIOZ Royal Netherlands Institute for Sea Research on the island of Texel. "It all ties together, because if an ancestor could form such protrusions, it could make a consortium of archaea and bacteria a lot more tight."

The Baums now estimate there's an 80% chance they're on the right track, and they're not the only ones gaining confidence. Ramanujan Hegde, a biochemist at the LMB who studies membrane proteins, is contributing to the upcoming seventh edition of the textbook *Molecular Biology of the Cell*. He and his colleagues decided that the Baum hypothesis will replace the phagocytosis-based model in the current edition. But there's still no proof, of course: Hegde is careful to use uncertain terms such as "could have".

Indeed, some others, including Gould, say the Baums' model doesn't fully explain how those membrane protrusions could have evolved into sheets, closed around the cell to create a complete outer boundary or acquired the characteristics of bacterial membranes. To explain the bacteria-like membranes, Gould and his colleagues have developed a model based on the fact that both free-living bacteria and mitochondria regularly release vesicles. They proposed in 2016 that the proto-eukaryote first acquired mitochondria – their theory doesn't specify how – which oozed vesicles into the cell. These vesicles provided the membrane materials that the evolving eukaryotic cell used to build its inner structure and external border[9]. This would explain why eukaryotes' membranes look like bacteria's, says Gould.

These and other competing models could be either supported or refuted as researchers continue to culture and study archaea; dozens of the microbes have now been grown successfully in the lab. Buzz Baum and his collaborators are investigating symbiosis in archaea and analysing microbial family trees to test their idea further. Nobu and his colleagues are investigating the protrusions in more detail and working on other Asgard archaea.

There might be more evidence waiting to be found. For example, the Baums predict that it might be possible to discover eukaryotes in which the tentacle membranes haven't quite disconnected from the exterior cell membrane, corresponding to an intermediate in their theory. What's looking more and more likely, at least, is that we owe our existence to an ancient love story of sorts between an archaeon and a bacterium. "We are part bacteria, part archaea, part new inventions," says Buzz Baum. "It's better together."

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