

Life force: why energy shapes evolution

Nick Lane

(University College London, UK)

Life on earth began some 4 billion years ago, but then got stuck at the level of bacteria for more than 2 billion years. The complex 'eukaryotic' cell arose abruptly in a singular event around 1.5–2 billion years ago. All eukaryotes share a long list of complex traits, from the nucleus to sex and senescence, which are all but unknown in bacteria. Why are humans so similar to mushrooms at the level of cells, even though we live so differently? Why did evolution follow such a peculiar trajectory? The answers might lie in the equally strange mechanism by which all cells generate ATP: chemiosmotic coupling.

Peter Mitchell won the Nobel Prize for his chemiosmotic hypothesis in 1978. The prize symbolically brought to an end two decades of intellectual turbulence, known as the 'ox phos wars'. Mitchell's idea was simple enough, at least in concept, but was wholly unanticipated. Far from there being some reactive chemical intermediate that coupled the energy released in respiration to ATP synthesis, Mitchell showed that the missing link was, in fact, an electrochemical proton gradient across a membrane¹. The idea has been lauded as an exemplary paradigm shift, the most counterintuitive idea in biology since Darwin, and the only one to compare with the ideas of Heisenberg, Schrödinger and Einstein.

Mitchell considered himself to be a physiologist, and from the outset was interested in how bacteria keep their insides different from their outside – how they maintain differences in ion concentration across their plasma membrane. Over the following decades, however, Mitchell's broad philosophical outlook became subsumed by more detailed and practical questions: how did the massive protein complexes embedded in the membrane physically pump protons from one side to the other? And the glittering question to top them all: how did the ATP synthase draw on this pent-up reservoir of protons to power ATP synthesis? John Walker's elucidation of its structure, a stunning rotating motor turning at over 100 revolutions a second, earned him a Nobel Prize in 1997². Since then, advances in crystallography and electron cryomicroscopy have illuminated the mechanisms of the respiratory complexes at nearly atomic resolution, notably complex I with its piston resembling a steam engine³.

But the success of structural biology has concealed our ignorance of the questions that motivated Mitchell: why cells work in this peculiar way. Mitchell certainly had a clear idea of the fundamental importance of ionic gradients – he presented a paper on the origin of life in

Moscow in 1957, four years before first publishing his chemiosmotic hypothesis – yet even he could hardly have imagined that membrane bioenergetics would turn out to be as universally conserved across life as the genetic code itself. This deep, deep conservation suggests that chemiosmotic coupling must have arisen very early in evolution, but the forces that drove its emergence are unknown, as are its effects on later evolution. Theoretical work now suggests that the requirement for proton gradients could have shaped the whole trajectory of evolution, from the origin of life to the divergence of archaea and bacteria, the singular origin of complex eukaryotic cells, and our own lives and deaths⁴.

Two paradoxes

The paradox at the heart of chemiosmotic coupling relates the interdependence of the three major components of respiration: proton pumps, impermeable membranes and 'turbines' such as the ATP synthase, which power work. What could have been the advantage of pumping protons across a membrane in the absence of an ATP synthase (or equivalent protein) that could harness the gradient? What was the point of having an ATP synthase if there were no proteins that could generate a proton gradient in the first place? And what use would any of these proteins have been if the membrane itself was leaky to protons, so they just slipped back through the membrane, short-circuiting the ATP synthase altogether?

The answers to these questions might lie in a second paradox: the startling differences between the two prokaryotic domains of life: the bacteria and the archaea. The revolution in phylogenetics over the last decade has shown that the deepest branch in the tree of life lies between the bacteria and the archaea, with the eukaryotes being a chimaeric derived domain⁵. The fact

that the genetic code is universally conserved, along with the ‘informational’ genes involved in transcription and translation, bits of the tricarboxylic acid cycle and amino acid biosynthesis, and chemiosmotic coupling, makes it clear that the bacteria and archaea share a common ancestor: the last universal common ancestor, or LUCA⁶. But the differences between the two groups are radical. Their cell membranes are fundamentally different in composition, as are their cell walls⁷. Central pathways of metabolism such as glycolysis are genetically distinct in bacteria and archaea⁷. Even the genes involved in DNA replication are not homologous in the two groups⁸. In a nutshell, returning to our main theme, membrane bioenergetics are universal, but membranes are not⁹. What sort of a cell could the LUCA have been?

Origins of life in alkaline hydrothermal vents

There is a simple and beautiful resolution to this paradox: life originated in an environment that had natural proton gradients. One such setting, known as an alkaline hydrothermal vent (Figure 1), was proposed as the ideal hatchery for life by the pioneering geochemist Mike Russell in the late 1980s, and developed in a series of theoretical and experimental papers since then^{10,11}, some of the most significant in collaboration with the biochemist Bill Martin^{7,12}. The essence of their ideas is as follows. In the absence of oxygen, at the origin of life, alkaline vents acted as electrochemical flow reactors: warm alkaline fluids saturated in hydrogen gas percolated through an interconnected labyrinth of micropores with thin catalytic walls containing iron sulfide minerals. These hydrothermal fluids mixed inside the vent with cool mildly acidic ocean waters, saturated in carbon dioxide. Such conditions are theoretically capable of driving the reaction of hydrogen with carbon dioxide to form organics¹³ and concentrating them to extreme levels within the pores of the vents¹⁴. This is not the place to discuss alkaline vents in detail. Suffice to say that they would have been common on the early Earth, and indeed throughout the cosmos, as their existence depends on but a handful of ingredients: the mineral olivine (abundant in the upper mantle of the earth and other planets), water and carbon dioxide⁴. Rock, water and CO₂. That’s it.

Let’s just grant that these alkaline hydrothermal vents could have given rise to the first cells with genes and proteins. Something did, and we and others are actively testing this hypothesis in the laboratory¹⁵. But even if true, the question remains: how could these early cells be powered by natural proton gradients? Without active pumps to expel the protons entering the cell, the entire system should have gummed up in electrochemical equilibrium in seconds. The most

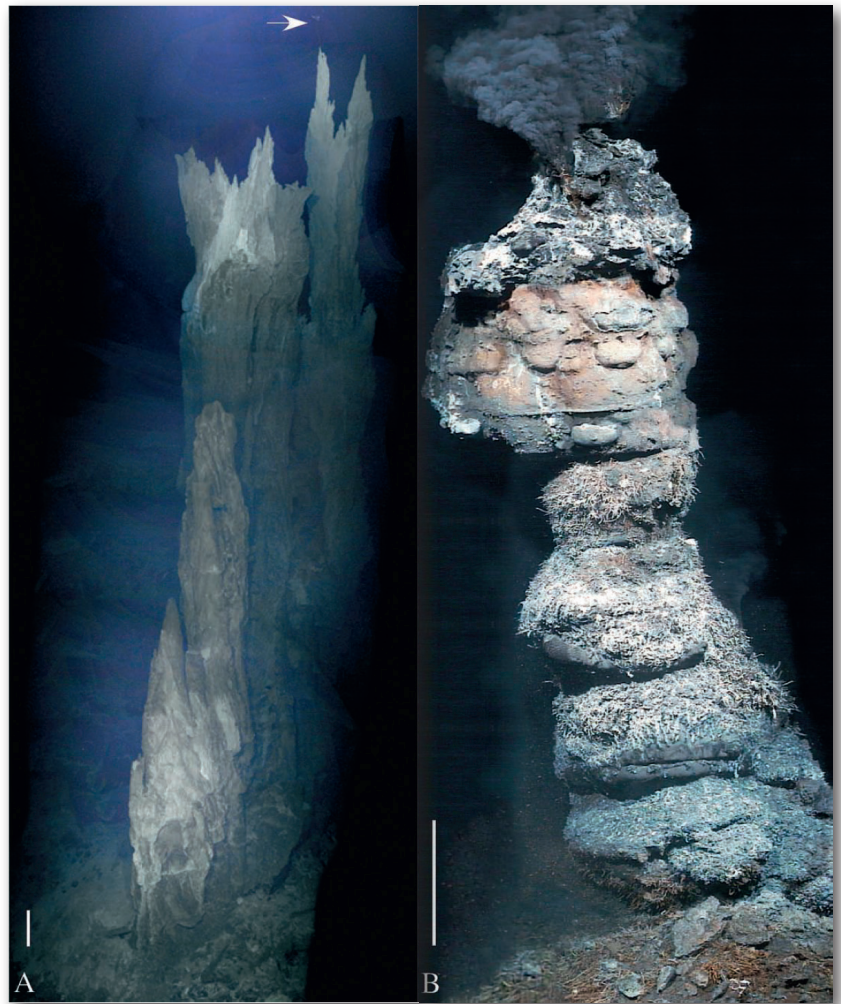


Figure 1. Comparison of an active alkaline hydrothermal vent at Lost City (a) with a black smoker (b). Scale bar, 1 m. Alkaline vents can stand as much as 60 m tall, equivalent to a 20-storey building. The white arrow at the top marks a probe fixed to the top of the alkaline vent. The paler regions of alkaline vents are the most active, but, unlike black smokers, alkaline hydrothermal fluids do not precipitate as ‘smoke’. Image courtesy of Deborah Kelly, University of Washington, USA, and the Oceanography Society.

compelling solution is that cells could only take advantage of the natural gradient if they had membranes that were extremely leaky to protons and hydroxide ions¹⁶. Then the protons that rushed in through the protein pores in the membrane (such as the energy-converting hydrogenase that methanogens use to drive carbon fixation, or the ATP synthase) could be neutralized or simply leave again passively down the proton gradient. Computational modelling supports this idea: a vent-bound LUCA could theoretically have driven both carbon and energy metabolism in much the same way as a modern methanogen, using natural proton gradients, but only if its membranes were extremely leaky to protons⁹. That rules out the incorporation of glycerol phosphate headgroups (which restrict proton

What makes us human?

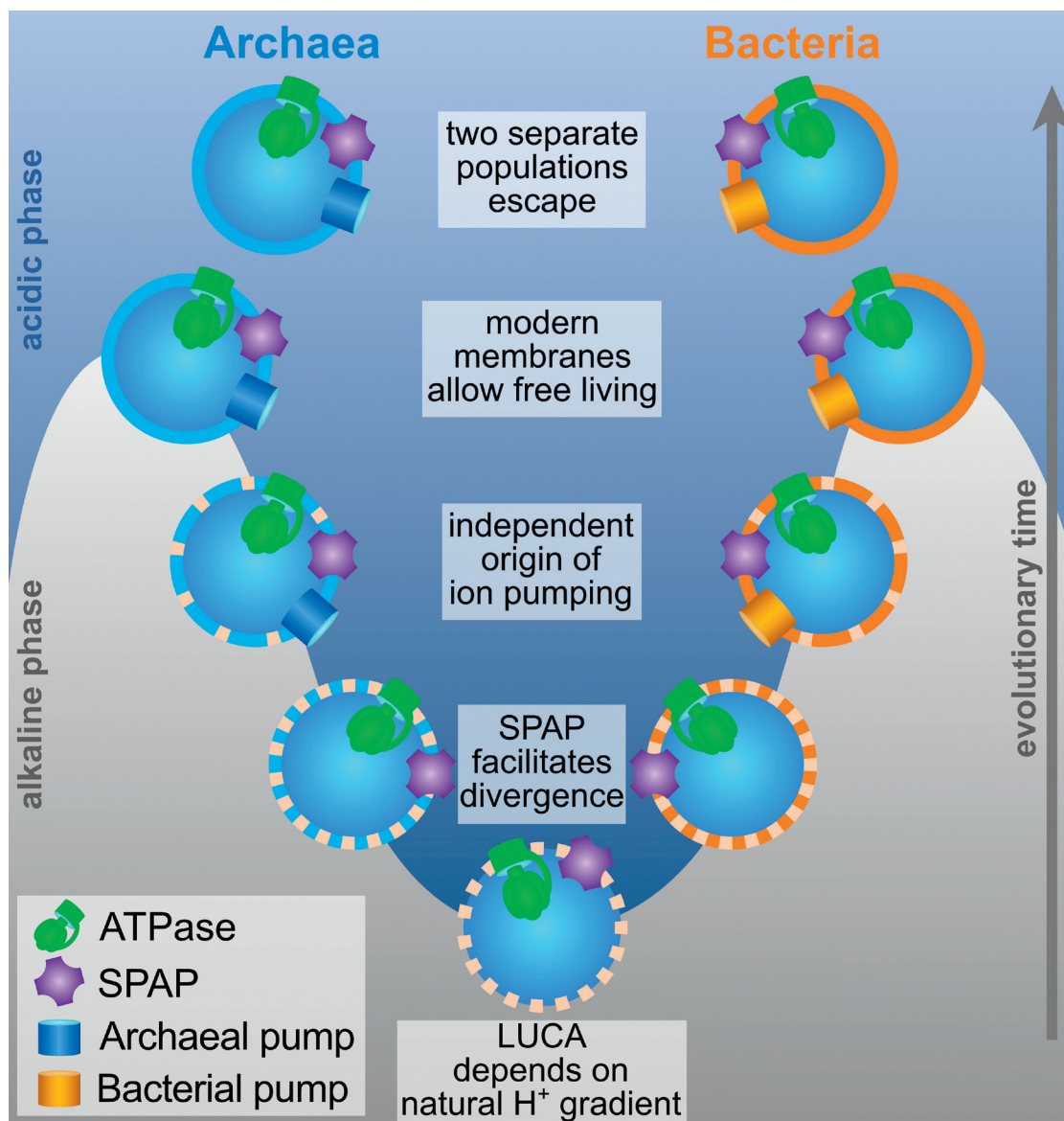


Figure 2. Independent escapes of bacteria and archaea from dependence on natural proton gradients in alkaline hydrothermal vents. Cells can only survive on natural proton gradients if they have leaky membranes, but active pumping across leaky membranes is futile. The evolution of a sodium/proton antiporter (SPAP) adds a biochemical sodium gradient to the geochemical proton gradient, giving cells more power. That allows cells to survive on lower gradients, facilitating spread and divergence into distinct populations. Equally importantly, SPAP gives a selective advantage to pumping even across a leaky membrane, arguably driving the evolution of distinct membrane pumps and membrane lipids in bacteria and archaea. From Sojo et al. (2014).

permeability) and could explain why the archaea and bacteria adopted distinct stereoisomers of glycerol phosphate in their membranes later on. A detailed analysis of free energy availability for a vent-bound LUCA with leaky membranes shows that there are tight constraints on the possible pathways to a free-living existence⁹, and the bacteria and archaea appear to have evolved independently from a common ancestor living in vents under parallel selective constraints (Figure 2).

Whatever the truth might be, and something has to explain the fundamental differences between bacteria and archaea, the fact remains that essentially all prokaryotes are chemiosmotic. They all share a common operating system, not only the genetic code, but also a membrane bioenergetic module that can plug in slightly different protein cassettes to allow the use of alternative electron donors and acceptors. Many of these (such as methane and sulphate) do not release enough energy to power

ATP synthesis by normal stoichiometric chemistry, but, because the redox reaction can be repeated many times, each time pumping a few protons across a membrane, chemiosmotic coupling allows cells to 'save up small change', and put it towards ATP synthesis, what we might call sub-stoichiometric energy conservation. The remarkable versatility of membrane bioenergetics allows cells to eke out a living under virtually any conditions, explaining the extraordinary adaptability of bacteria and archaea. But, while favouring metabolic diversity, chemiosmotic coupling also limits the morphological complexity of prokaryotes⁴.

The astonishing benefits of mitochondria

All morphologically complex life on Earth is composed of eukaryotic cells, cells with a 'true' nucleus and all kinds of internal membranes and organelles. On average, eukaryotic cells are at least 15 000-fold larger than prokaryotes, with genomes to match¹⁷. The largest bacterial genomes are about 12 Mb, among the cyanobacteria, whereas eukaryotic protists range up to 100 000 Mb or more, again four or five orders of magnitude greater. Not only that, but eukaryotes share a long list of traits essentially unknown in either bacteria or archaea. This is peculiar, to put it mildly. The last eukaryotic common ancestor (LECA) was a fully-fledged eukaryotic cell, with a nucleus, straight chromosomes, mitosis and meiosis, sex, introns and exons, a nuclear membrane with nuclear pore complexes, dynamic cytoskeleton, motor proteins, endoplasmic reticulum, lysosomes, Golgi apparatus, mitochondria, you name it: the works⁴. Bacteria and archaea show little tendency to evolve any of these traits in a comparable form. There are no surviving evolutionary intermediates, nothing to tell the tale, and no agreement on how or why all these eukaryotic traits evolved.

But there are clues. All eukaryotes have mitochondria, and all mitochondria (at least those that can still respire) always retain a small bioenergetic genome. There's no real consensus as to why, but the biochemist John Allen argues that these tiny genome outposts are required to control respiration in mitochondria and chloroplasts¹⁸. It would be extraordinary if this were not true. Mitochondria have a membrane potential of 150–200 mV across a membrane that is 5–6 nm thick, giving a field strength of 30×10^6 V/m, equivalent to a bolt of lightning. Surely that requires special measures. But the same reasoning should apply not only to eukaryotes with mitochondria but also to giant bacteria (of which there are a few), or those with convoluted inner membranes. If subsidiary genomes really are needed to control respiration, then these giant bacteria should have multiple genomes. They do – so many that it's known as extreme polyploidy, with

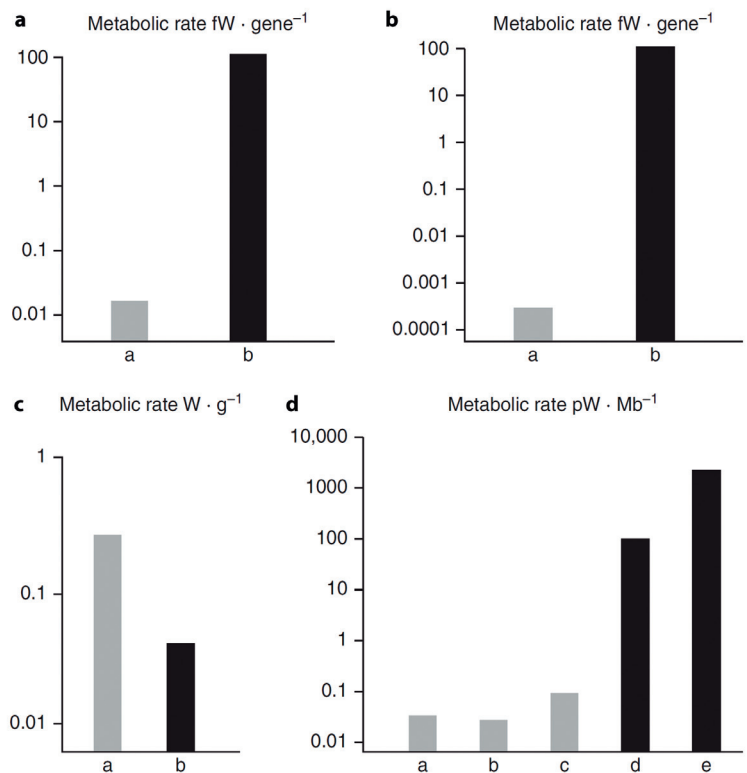


Figure 3. (a) Mean metabolic rate per gene in bacteria (a, grey bar) compared with eukaryotes (b, black bar), when equalized for genome size. (b) Data equalized for cell volume (15 000-fold larger in eukaryotes) as well as genome size. A single eukaryotic cell has ~100 000-fold more energy per gene than a single bacterial cell scaled to eukaryotic size. (c) Mean metabolic rate per gram in bacteria (a, grey bar) compared with eukaryotes (b, black bar). Per gram, bacteria respire about 3 times faster than eukaryotes. In other words, the differences in energetics between bacteria and eukaryotes relate to their differences in cell volume and membrane structure, not to flux rates per gram of protein. (d) Metabolic rate per haploid genome, taking into consideration genome size, copy number (polyploidy) and cell volume. In this case, a is *Escherichia coli*, b is *Thiomargarita*, c is *Epulopiscium*, d is *Euglena* and e is the large *Amoeba proteus*. Original data from Lane and Martin (2010), *Nature*.

Epulopiscium having as many as 200 000 copies of its complete genome. As Bill Martin and I showed¹⁷, taking all of these genomes into consideration gives eukaryotes at least 100 000-fold more energy per gene compared with even the most energetic bacteria (Figure 3).

The real benefit of mitochondria is not that they respire oxygen – plenty of bacteria can do that too – but that they have lost almost all their genes. Eukaryotes don't have more DNA in total than giant bacteria, but have radically altered its distribution: all eukaryotes have tiny bioenergetic genomes, which support energetically a massive nuclear genome. The true signature of eukaryotes is not the nucleus alone, but this extreme genomic asymmetry. Just consider the energy savings. Think of a eukaryotic cell with 100 bacterial endosymbionts, each one of which has lost 5% of its genome, say 200 genes that it no longer needs in the

What makes us human?



Figure 4. Prokaryote or eukaryote? This cell looks superficially like a eukaryote with a cell wall (CW), plasma membrane (PM) and nucleus (N) surrounded by a nuclear membrane (NM). It has several endosymbionts (E) that look like hydrogenosomes. It's quite big (10 μm in length), and the nucleus is large, taking up nearly 40% of the cell volume. But the nuclear membrane is a single layer, not a double membrane. There are no nuclear pore complexes, just occasional gaps. There are ribosomes in the nucleus (mottled grey regions) and outside the nucleus. The nuclear membrane is continuous with other membranes including the plasma membrane. DNA is in the form of thin filaments, 2 nm in diameter as in bacteria, not eukaryotic chromosomes. Could this be an endosymbiosis between prokaryotes that is recapitulating eukaryotic evolution? Courtesy of Masashi Yamaguchi.

cytoplasm of its host cell. If each gene is normally expressed in 2000 copies, and each protein has an average of 250 amino acids, the energy savings from *not* expressing those genes is 50 billion ATP molecules¹³. Over a lifecycle of 1 day, that's 580 000 ATP molecules per second, enough to power the *de novo* synthesis and assembly of 4 μm of actin every second! Mitochondria didn't lose just 5% of their genomes, but 99%; and there can be as many as 300 000 mitochondria in large amoebae. There's no requirement for these colossal energy savings to be spent on gene expression or sustaining a giant nuclear genome, but that's what does happen.

A singular origin of complexity

Endosymbiosis, a symbiotic relationship in which one organism lives inside the other, was pivotal to the origin of the eukaryotes. Without mitochondria, cells just can't become large and complex. But why don't all kinds of cells acquire mitochondria then? It's difficult. The pioneering phylogenetic work of Martin Embley and colleagues shows that the host cell that acquired bacterial endosymbionts was an archaeon⁵. That means it was a prokaryote, i.e. lacking a nucleus and all the other eukaryotic paraphernalia. That has massive implications. The host cell was not some kind of primitive phagocyte, whose properties can't be defined in any meaningful

way, but a morphologically simple cell, probably with an archaeal cell wall, and unable to simply phagocytose its symbionts. How they entered is still a mystery, but we do know of one or two examples of bacteria living inside other bacteria that have a cell wall⁴, so we know it's possible, if very rare. Equally significantly, if the host cell was an archaeon, then all of that formidable list of eukaryotic traits must have arisen in the context of an endosymbiosis between two prokaryotes. What little phylogenetic evidence exists is consistent with this interpretation; the genes encoding the nucleolus and nuclear pore complexes are chimaeric, for example, with some deriving from bacteria (the endosymbiont) and others from archaea (the host cell)¹⁹.

This is a double whammy of an evolutionary bottleneck, and explains why complex life only arose once on Earth. Bacteria and archaea are constrained by their membrane bioenergetics, and, despite their metabolic versatility, show no sign of evolving complex morphological traits. The eukaryotes broke out of this eternal loop via an endosymbiosis between prokaryotes, which in itself is a very rare event, although, if we know of a couple of examples today, then it presumably happened on thousands or millions of occasions over 4 billion years of evolution. But that's only half the whammy. After that, they had to get along together, synchronizing life

cycles and resolving intimate conflicts. The long list of unique eukaryotic traits suggests this reconciliation was prolonged and difficult, but it also gives a compelling insight into why we are as we are.

Predicting the evolution of complex traits

Sex, two sexes the nucleus, the germline: all can be explained in terms of the requirement for these conflicting genomes in each and every eukaryotic cell⁴. The balance between their requirements can explain unanticipated trade-offs between aerobic fitness and fertility, sexual maturation and lifespan²⁰. All that's another story. But is any of it predictable? Can we explain eukaryotic physiology in terms of the interactions between mitochondria and their host cells? I think so, and, if I'm right, this could also give insights into what goes wrong in disease. Why, for example, mutant mitochondria can proliferate to take over whole tissues in cancer or aging; why 40% of pregnancies end in early 'occult' miscarriage; and why genome-wide association studies (GWAS) of diseases from diabetes to depression fail to account for the majority of the known heritable component. We are addressing some of these questions by normal population genetics, albeit starting from an unusual place, populations of cells within cells²¹. I hope these studies will make predictions that can be tested in the laboratory.

In the meantime, I have an image in mind, one that offers cautious grounds for optimism. It is a cell, a microbe found clinging to the back of a polychaete worm on the slopes of a deep sea hydrothermal vent off the coast of Japan²² (Figure 4). On first glance, it looks a lot like a eukaryote: it is about 10 µm long, with

a large nucleus taking up nearly 40% of the cytoplasm, endosymbionts resembling hydrogenosomes and internal membranes. But look again! This nucleus is not surrounded by a double membrane, but just one. The DNA is composed of thin filaments, 2 nm in diameter, like bacteria. There are ribosomes in the nucleus. The internal membranes look nothing like endoplasmic reticulum, lacking a lumen, and are continuous with the plasma membrane. So what is it then? I think it is a prokaryote that has acquired endosymbionts, and is recapitulating eukaryotic evolution. Not exactly the same, but pretty similar and for similar reasons: similar conflicts, similar resolutions. This cell is a riddle that holds the answer to life, the universe and everything! The only problem is that it's the only specimen ever found, and it was sectioned for electron microscopy. We'll just have to wait for another one. Don't hold your breath. ■



Nick Lane is a Reader in Evolutionary Biochemistry in the Department of Genetics, Evolution and Environment at University College London. He leads the UCL Research Frontiers Origins of Life programme and was a founding member of the UCL Consortium for Mitochondrial Research. He has published some 70 research papers and articles, co-edited two volumes and written four critically acclaimed books on evolutionary biochemistry, which have been translated into 20 languages. Nick was awarded the Royal Society Prize for Science Books for Life Ascending in 2010, and received the Biochemical Society Award in 2015. His latest book *The Vital Question* was published in April 2015. email: nick.lane@ucl.ac.uk

References

1. Mitchell, P. (1961) *Nature* **191**, 144–148
2. Abrahams, J.P., Leslie, A.G., Lutter, R. and Walker, J.E. (1994) *Nature* **370**, 621–628
3. Baradaran, R., Berrisford, J.M., Minhas, S.G. and Sazanov, L.A. (2013) *Nature* **494**, 443–448
4. Lane, N. (2015) *The Vital Question: Energy, Evolution and the Origins of Complex Life*, WW Norton, New York
5. Williams, T.A., Foster, P.G., Cox, C.J. and Embley, T.M. (2013) *Nature* **504**, 231–236
6. Martin, W., Sousa, F.L. and Lane, N. (2014) *Science* **344**, 1092–1093
7. Martin, W. and Russell, M.J. (2003) *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **358**, 59–83
8. Leipe, D.D., Aravind, L. and Koonin, E.V. (1999) *Nucleic Acids Res.* **27**, 3389–3401
9. Sojo, V., Pomiankowski, A. and Lane, N. (2014) *PLoS Biol.* **12**, e1001926
10. Russell, M.J., Daniel, R.M., Hall, A.J. and Sherrington, J. (1994) *J. Mol. Evol.* **39**, 231–243
11. Russell, M.J. and Hall, A.J. (1997) *J. Geol. Soc. London* **154**, 377–402
12. Martin, W. and Russell, M.J. (2007) *Philos. Trans. R. Soc. London B Biol. Sci.* **367**, 1887–1925
13. Lane, N. (2014) *Cold Spring Harb. Perspect. Biol.* **6**, a015982
14. Baaske, P., Weinert, F.M., Dühr, S. et al. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104**, 9346–9351
15. Herschy, B., Whicher, A., Camprubi, E. et al. (2014) *J. Mol. Evol.* **79**, 213–227
16. Lane, N. and Martin, W. (2012) *Cell* **151**, 1406–1416
17. Lane, N. and Martin, W. (2010) *Nature* **467**, 929–934
18. Allen, J.F. (2003) *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **358**, 19–37
19. Mans, B.J., Anantharaman, V., Aravind, L. and Koonin, E.V. (2004) *Cell Cycle* **3**, 1612–1637
20. Lane, N. (2011) *Science* **334**, 184–185
21. Hadjivasilou, Z., Lane, N., Seymour, R. and Pomiankowski, A. (2013) *Proc. R. Soc. Lond. B Biol. Sci.* **280**, 20131920
22. Yamaguchi, M., Mori, Y., Kozuka, Y., et al. (2012) *J. Electron Microsc.* **61**, 423–431