Newsletter of the NZ Biochemistry and Molecular Biology Society

SOUTHERNBLOT

SUMMER 2015/2016

THIS ISSUE

What's on for 2016

Anthony Poole, NZSBMB President

It's nearly the end of the year, but your NZSBMB exec are already working away on plans that we hope will make 2016 an exciting and busy year for the Society. I want to bring your attention to several things in the pipeline and, as always, look forward to receiving your thoughts on any or all of these.

QMB 2016

We're committed to being involved in the 2016 Nelson Molecular Biology meeting (for those that don't know, QRW is going on tour as the usual venue in Queenstown is being refurbished). There have been a number of new ideas that previous QMB organisers have brought to the table, so we'll be learning from them while adding some of our own ideas to the mix – we're confident the Society will help run a successful event in Nelson.

NZMS/NZSBMB 2016

In November 2016 we'll be running a joint annual conference with the NZ Microbiological Society. The 2014 meeting in Wellington was a great success for our Society, and there was good feedback on its value for the community, so we're again joining forces to bring you the NZMS/NZSBMB 2016 meeting in Christchurch. This will coincide with the height of activity around the Christchurch rebuild, so as well as great science, it will provide a unique opportunity for those of you who live elsewhere in the country to witness the new Christchurch emerging from the rubble of the old.

A Federation of Societies in the Life Sciences

At the recent meeting for Constituent Organisations of the Royal Society, a number of societies, ours included, started discussing the idea of a Federation, modelled loosely around FASEB, the Federation of American Societies for Experimental Biology. These discussions are in their infancy, but there are at least 8 societies that are interested in exploring this avenue. Some of the reasons we might want to think seriously about this are obvious. NZ has many small societies, each with modest funds and a small-to-medium membership. This means most of us struggle to devote funds to anything larger than small student travel scholarship funds. The idea of funding larger initiatives is on all our collective radars, but is still just a pipe dream. There's also a lot of duplication of effort! It's also evident that many of us could (and indeed some of us do) belong to more than one society, as our interests are broader than the boundaries of a single society. That said, many of us are not that keen to join (and pay subs to) half a dozen societies, spanning general science and our own varied research interests. So putting our heads together and thinking about how we might contribute to building a stronger science system seems like an important, and timely, endeavour. If you have any thoughts or advice to offer the Society on this initiative, do let me know!

Membership for Technical staff

We all know that the glue that holds any good lab together is its technical staff. They are the unsung heroes of science, making sure our labs run well, our students and postdocs are properly trained, and doing a million other things not in their job description. We would therefore like to extend a warm welcome to the nation's technical experts to join NZSBMB, and we'd like to hear what would make you want to become members. Some of the things we're thinking about developing are dedicated events for technical staff at our conferences, which will aim to foster networking and learning new skills. As part of that, we're planning to introduce a special technical staff membership rate and financial support to attend conferences through disbursement of a travel stipend.

New Newsletter Editor

Last but not least, we're delighted to welcome Astra Heywood onto our team as the new Newsletter Editor. Astra is a PhD student at Otago, and also has professional experience in web design, so her talents will be very valuable as we continue to think about ways to improve the Society's reach and relevance. As you can see, she's already been hard at work giving Southern Blot a brand new look. Welcome Astra! ■

Wishing you all the best for a wonderful summer break!

ANT

Email: anthony.poole@canterbury.ac.nz
Twitter: @pouletm

Kathryn Stowell

Associate Professor in Biochemistry

Institute of Fundamental Sciences
Massey University

Describe your research in one sentence.

Screening for genetic variants associated with Malignant Hyperthermia (MH), demonstrating genotype/phenotype correlation and functionally characterising those that segregate.

What gets you up in the morning?

My alarm clock most mornings! In general I still enjoy my job and those days that I know I can get into the lab I walk to work with a spring in my step, knowing that I will be doing what I enjoy most and communicating directly with my lab people. We work hard but we have fun while we are at it.

What are you doing right now?

Aside from writing this, I am filling in time between the end of the first day of the biennial HGSA (NZ branch) in Christchurch before heading out for the conference dinner at the Pegasus Arms. I seldom attend this meeting and I was impressed with how far molecular genetic analysis has progressed in both predictive screening and diagnosis. It is also very heartening that it is not just in my very specialised field that as we can gain more information, the interpretation of results gets more complicated.

Do you know your h-index?

I have a vague idea but in my opinion these are over-rated and often not a true reflection of what they claim to be. I have always thought that rewards would come by being honest and working hard. In my case I think that I have been rewarded with a very mediocre h-index, even though the successes and recognition have come relatively infrequently and have taken longer than they might have. I still get satisfaction about doing a good job whether it is in teaching, administration or research. It is what being an academic is all about.

What bit of research has caught your eye recently?

Perhaps not just lately but the two high-resolution Cryo-EM structures of RyR1 and RyR2 that were published earlier this year are pretty amazing. In some regions of these >2MDa proteins, we can actually see the amino acids!!!!

How did you get into science?

Ever since I was ten years old I had my heart set on medical school. Having got to the middle of the second year at Otago Med School I found that anatomy and physiology were not for me, and the only course I really enjoyed was biochemistry!!! Feeling that I had had enough of being a student I got a job as a technician in a biochemistry lab. My career progressed naturally from there albeit rather circuitously and largely part time.

What would you have done if you didn't go into science?

I don't think there was ever another option. If med school was more like it is today I may well have stayed and completed the MB, ChB. The only other thing that I really enjoyed was playing the flute and that never appealed to me as a career option and would have been even less lucrative than science.

What do you consider to be the highlight of your career so far?

There have been several, but the one that always comes to mind when I am asked this question was an occasion in 1998 just after we had identified the first genetic variant in RYR1 that cosegregated with MH-susceptibility. A young man of our largest MH-susceptible family was due for surgery. We had just developed the DNA test for his familial variant and carried out the test the day before he was scheduled for a diagnostic muscle biopsy and in vitro contracture test. After one failure we managed to get the result through in time. He carried the variant and therefore was MH-susceptible so the muscle biopsy was cancelled. (Just to be clear here, the muscle biopsy takes several grams of muscle! It is effectively a lump of meat.) It was the first time that I felt that what we were doing was actually going to make a significant difference to people and their healthcare.

If you were stranded on a desert island, what three biochemicals and/or biochemists would you want with you?

It is hard to identify who is and who is not a biochemist these days, but I think I would enjoy conversation with Carl Wittwer and he would be able to build me a real-time PCR machine from flotsam. Steve McKnight would remind me that I used to work in transcriptional regulation and I would not want to go anywhere without some HRM mastermix! ■

Evert Loef

European conference of Immunology, Vienna, Austria, September 2015

University of Auckland

The European congress of Immunology (ECI) is held every three years. The ECI 2015 was the 4th ECI and it was held in Vienna in Austria and it was opened with different musical pieces from famous Viennese composers performed by a full orchestra in typical Viennese fashion. The congress had over 3500 pre-registered participants. Despite the number of people and big number of presentations and different sessions the conference was very well organized with all sessions (that I have attended) running exactly on time.

According to the statistics in the abstract book there were more than 3000 accepted abstract submissions for posters and oral presentations. From all those abstracts 2 were from New Zealand (Compared to 74 from Australia). One of those 2 abstracts (and the only oral presentation from a New Zealand based university) was mine.

I presented on some of the work I have been doing for the last 3 years of my PhD. The main focus of my research is human T cell activation and specifically the role of proteases and their specific inhibitors in T cell-T cell interaction and T cell proliferation following activation. Even though I work with T cells (a type of immune cell) my work is very molecular and cellular based.
The conference was buzzing with researchers that are fully dedicated to immunology and this resulted in typical immunology type questions following my research presentation regarding if a knockout mouse model is available for my work or if we looked at different specific T cell subsets. Immunologists love their knockout mice and different immune cell subsets, the more the better.
At the end of the first conference day there was a workshop on scientific writing. During the workshop the editor of the European Journal of Immunology supplied us with inside information on what is important in a paper from and editor's point of view. This was of particular interest to me because I am nearing the end of my PhD and am trying to publish my research before I start writing my thesis. In the early stages of our education as a scientist a lot of focus is placed on knowledge and how to do research but the skills of presenting our work in written or oral form do often not receive sufficient attention. Some of the tips on how to write different parts of a paper seems straight forward but are often forgotten when writing and using other research papers as examples.

A big part of the congress was dedicated to tumour therapy and specifically on T cell exhaustion. T cell exhaustion is a molecular process whereby the tumour cell is able to deregulate the T cell in such a way that is unable to attack the tumour and kill it. By reversing this mechanism it would be potentially possible to stop the growth and reduce the tumour (sometimes even completely eradicating it!). This research is still in the early stages but there is already some tremendous success. At the moment there is a lot of research ongoing looking for different molecular targets that can be manipulated to reverse this exhaustion process.

Potentially the proteases and their inhibitors that I am investigating could play a role during T cell exhaustion. So it was great to see all this ground-breaking new research at these vital early stages from the world top laboratories at this conference. I am very grateful that the financial support that the NZBMB provided made this possible.

The conference dinner was held in Grumpoldskirchen a little very picturesque town about 45 min out of Vienna with less than 4000 people (about the same number of people that attended the conference!). When we arrived at the town we were greeted by a classic Austrian fanfare and inside the town square locals were performing a local dance. Grumpoldskirchen is very famous for its heurigers (wine taverns). There we were supplied with the famous local wine and rustic Viennese dishes which included different types of roast meat, sauerkraut, potatoes and breads with different soft spreadable cheeses.

In the end I would like to thank all the funding bodies that made this research and attendance to the conference possible: The New Zealand Society Biochemistry and Molecular Biology for providing me the travel grant, The University of Auckland for providing me with a scholarship and the Marsden Fund of the Royal society of New Zealand for supporting the research.

Nikola Palevich

Australian Society for Microbiology Annual Scientific Meeting 2015, Canberra, July 2015

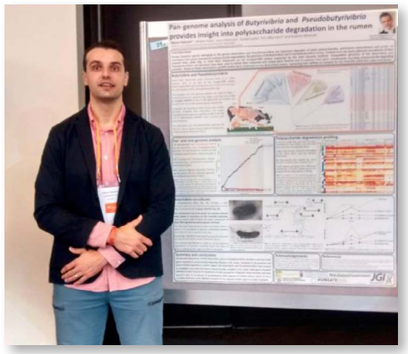
Massey University

The ASM2015 was the 43rd Annual Scientific Meeting for the Australian Society for Microbiology and was held at the QT in Canberra. For this conference I presented a poster on my PhD project entitled "Pan-genome analysis of *Butyrivibrio* and *Pseudobutyrivibrio* provides insight into polysaccharide degradation in the rumen". The conference theme was "One Microbiology" that was designed to celebrate microbiology in all environments regardless of purpose and to recognize the profound importance of microbiology and microbiologists to the future of human and environmental health on Earth.

The most relevant and outstanding talk was done by Janet Jansson who came all the way from the USA. Janet is an acclaimed microbial ecologist and delivered the Rubbo Oration, on the employment of state-of-the-art and novel "omics" approaches on large and complex meta-omic datasets to determine relationships between diet and microbial communities of the human gastrointestinal tract. As part of this years program, students and early researchers were invited to a host of career advancement events including the Nancy Mills Student Mentoring Breakfast and Lunch. I was fortunate enough to be paired with Janet at both of these events and we got to know one another's research topics and goals quite well. During the poster session Janet was kind enough to come over, which was great because others followed her lead and I was fortunate enough to have the opportunity to talk about the work I do and where I'm from to some of the most influential international gut microbiologists in the world. In that respect the networking opportunity from attending the ASM2015 was excellent!

NZSBMB Travel Awards

The reason for attending this conference is because I was fortunate enough to win the ASM – NZMS Postgraduate Research Travel Award. This award allows for the reciprocal exchange of one student member each year to visit the National conference of the other society and to visit the research lab of a collaborating researcher in that country. The ASM-NZMS Postgraduate Travel Grant offered me an opportunity to travel to Dr Stuart Denman's laboratory in Brisbane, Queensland, Australia and stay for 14 days to conduct research. More specifically, the purpose of my stay was to conduct analyses on *Butyrivibrio* and *Pseudobutyrivibrio* genomes to characterise their hemicellulose-degrading ability. We believe this work will provide the first systematic, phenotypic, comparative genomic and functional analysis of ruminal *Butyrivibrio* and *Pseudobutyrivibrio* pan-genomes, which will not only define their conserved features involved in hemicellulose degradation, but also begin to differentiate their unique gene complements and growth characteristics that separate them as discrete genera and their strains as separate species. In the future, this information will be fundamental to understanding digestion of hemicellulose in the rumen and in maximising productivity in ruminant animals.



As well as presenting my *Butyrivibrio* and *Pseudobutyrivibrio* comparative genomics work at the ASM conference in Canberra in mid-July, this was also an opportunity to present my work at the CSIRO research facility at the Queensland Bioscience Precinct at the University of Queensland St Lucia campus in Brisbane. The main benefit of this opportunity was that I received peer feedback from acclaimed and emerging scientists which will be valuable prior to submission of my PhD thesis.

I sincerely wish to thank the Institute of Fundamental Sciences (IFS) and the New Zealand Society for Biochemistry and Molecular Biology (NZSBMB) postgraduate travel funds for supporting my travel costs. But most of all, I wish to express my gratitude to the New Zealand Microbiological Society (NZMS) for awarding me the "ASM-NZMS Postgraduate Travel Award" this year. It was truly a trip of a lifetime. ■

Ben Peters

ComBio2015, Melbourne, October 2015

University of Otago

The Combio conference was a large conference combining the annual meetings of five New Zealand and Australian societies. With 20 incredible plenary speakers, 8 concurrent symposiums and poster sessions, there was a huge variety of world class research being presented. Amongst these, I had the privilege of presenting my research during the systems biology symposium.

My presentation focused on the development of the male germ line in flowering plants. Development of the germline can be broadly broken into two stages, specification and differentiation. On the differentiation side, a lot is already known. Specifically we know that a particular transcription factor (DUO1) is turned on very early after specification, DUO1 in turn, controls most of the germ line differentiation stage. Therefore we used DUO1 to try find the key to germ line specification. The first stage of this is to identify what activates DUO1. Hypothesising that conservation of sequence is due to conserved function I looked for conserved regions in the promoter of DUO1. First within 345 *A. thaliana* accessions and then across 24 other flowering species. This analysis collectively revealed three motifs that I showed were necessary for the expression of DUO1. The next stage of my research is to find the transcription factors that bind to these regions.

My presentation was well received and I won the NZSPB best student speaker award for it. The value of sharing my work on such a platform was immeasurable. Through it I gained valuable feedback and ideas for future experiments. It was also incredibly useful for networking as other prominent scientists in my field of research learned of the work I was doing.

The other speakers at the conference often had well pitched presentations that allowed me to learn a large amount from areas that I was not familiar with before the conference. A number of these presentations gave me ideas for my own work that I would not have thought of unless I went to such a broad meeting.
I would encourage other students to try get to conferences like Combio. Particularly conferences with quality international speakers from outside your field as it forces you to broaden your horizons and become aware of what is happening in the wider scientific community. The specialist symposia were well themed so that they gave a great depth of understanding within a field through 5 different presentations from different lab groups and approaches.
Lastly I would like to thank NZSBMB for the travel grant that enabled me to come to this conference. It was a very valuable experince. ■

STUDENTS AND NEW POST-DOCS!

Do you want support for your next conference overseas?

Student Travel Awards are available to support travel to any international conference. Awards are up to NZ$1000 each, and are intended to assist post-graduate students to attend their first conference outside New Zealand.

In addition, we are now offering the Early Career Travel Award. This is in conjunction with the Biochemical Society (UK), and it is open to student members, as well as early career researchers (0-3 years post-PhD). These awards are specifically to enable attendance at Harden Conferences or Focused Meetings of the Biochemical Society.

Details of the awards, and the application form, are available at http://nzsbmb.science.org.nz/awards.html.

Astra Heywood

Editor of Southern Blot
Department of Biochemistry
University of Otago

Before becoming editor of Southern Blot, I was awarded an NZSBMB travel scholarship to attend Combio in Melbourne. I had travelled to Melbourne alone so was pleased to see Miriam (Miriam and I are in the same department) at the careers symposium. This year the symposium focused on careers outside of research and as someone who also has a background in design it was good to see the other career opportunities. After the symposium I mentioned my stint in design to Miriam, which eventually led to me being editor of Southern Blot.

Before Combio, I had taken a few days off in order to get to know Melbourne. I was staying in North Fitzroy with family and I was determined to master the public transport system before the conference began. This led to me seeing as much of Melbourne as possible which included visiting all the big galleries including the David Bowie exhibit, parks and the zoo. Despite seeing so much I only took a single photo of a Lemur.

Combio was the first "international" conference I have attended. Currently I am approximately half way through a PhD with a focus on proteolytic regulation in *Pseudomonas aeruginosa*.

I presented a poster on proteases that may be involved in directed proteolysis of the anti-sigma factor binding protein FpvR. FpvR is a membrane bound protein and it seems as though all the proteases involved in the degradation of FpvR are also membrane bound, so I thoroughly enjoyed the talk by Professor Martin Caffrey. It was also exciting to see super resolution images of *Pseudomonas* presented by Lynne Turnbull, visualising the uptake of DNA from neighbouring cells.

Combio Cocktail Party, From Left: Chris Brown, Nicole Shonrock, David Croucher, Astra Heywood, Miriam Sharpe

It was great to meet new people and Nicole Schonrock knows if she ever gets to Dunedin we will definitely take the horses out for a blast down the beach. Attending Combio was an invaluable experience where I not only got to learn about areas outside of bacterial molecular systems, I also got to see what is possible post-PhD.

I hope you enjoy this issue of Southern Blot and if you have any suggestions or contributions you would like to make, please let me know. ■

ASTRA
Email: astra.heywood@gmail.com

Combio2015

Melbourne Convention and Exhibition Centre
27 September to 1 October 2015

Career Development Forum co-convenors and speakers, from left. Front row: Julie Miland, Laura Zamurs, Jennifer Henry, Sarah Hennebry, Martin Elhay and Sam Richardson (co-convener). Back row: Ros Glead-ow (co-convener), Belinda Smith, Len Pattenden, Sarah Brooker, Nicole O'Leary and Jessica Lye.

ComBio2015 Chair, Marie Bogoyevitch, opens proceedings

Welcome Mixer and Cocktail Party

Student Mixer with International Speakers

Awards and Plenary Lectures

ASBMB Lemberg Medallist Professor Christina Mitchell

Professor Martin Caffrey

Plenary Lecturer Professor Minoru Yoshida

From left: NZSBMB Secretary Associate Professor Kerry Loomes, NZSBMB Custom Science Awardee Associate Professor Peter Fineran and Andrew Kyriazis of Custom Science

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 membrane[1]. 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 1997[2]. 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 engine[3].

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 deaths[4].

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 domain[5]. 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 LUCA[6]. But the differences between the two groups are radical. Their cell membranes are fundamentally different in composition, as are their cell walls[7]. Central pathways of metabolism such as glycolysis are genetically distinct in bacteria and archaea[7]. Even the genes involved in DNA replication are not homologous in the two groups[8]. In a nutshell, returning to our main theme, membrane

bioenergetics are universal, but membranes are not[9]. 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 organics[13] and concentrating them to extreme levels within the pores of the vents[14]. 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 dioxide[4]. Rock, water and CO_2. 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 laboratory[15]. But even if

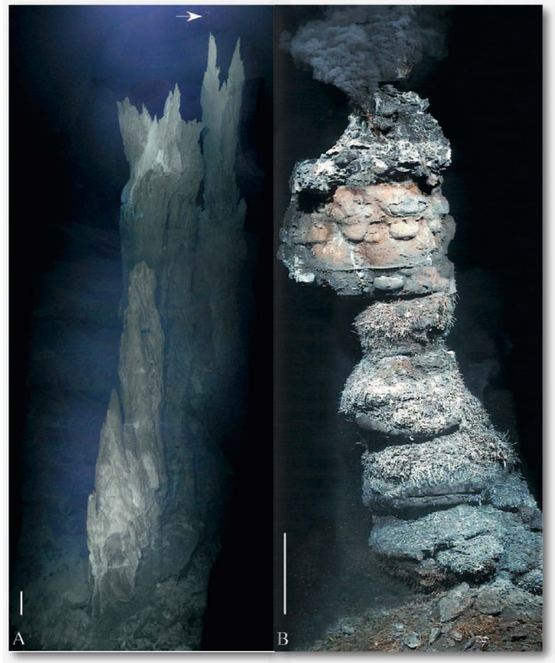


Figure 1. Comparison of an active alkaline hydrothermal vent at Lost City (a) with a black smoker (b). Scale bar, 1 m. Alkaline vents can stand as much as 60 m tall, equivalent to a 20-storey building. The white arrow at the top marks a probe fixed to the top of the alkaline vent. The paler regions of 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.

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 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 ions[16]. 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 protons[9]. That rules out the incorporation of glycerol phosphate headgroups (which restrict proton 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 existence[9], 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 prokaryotes[4].

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, eukary-

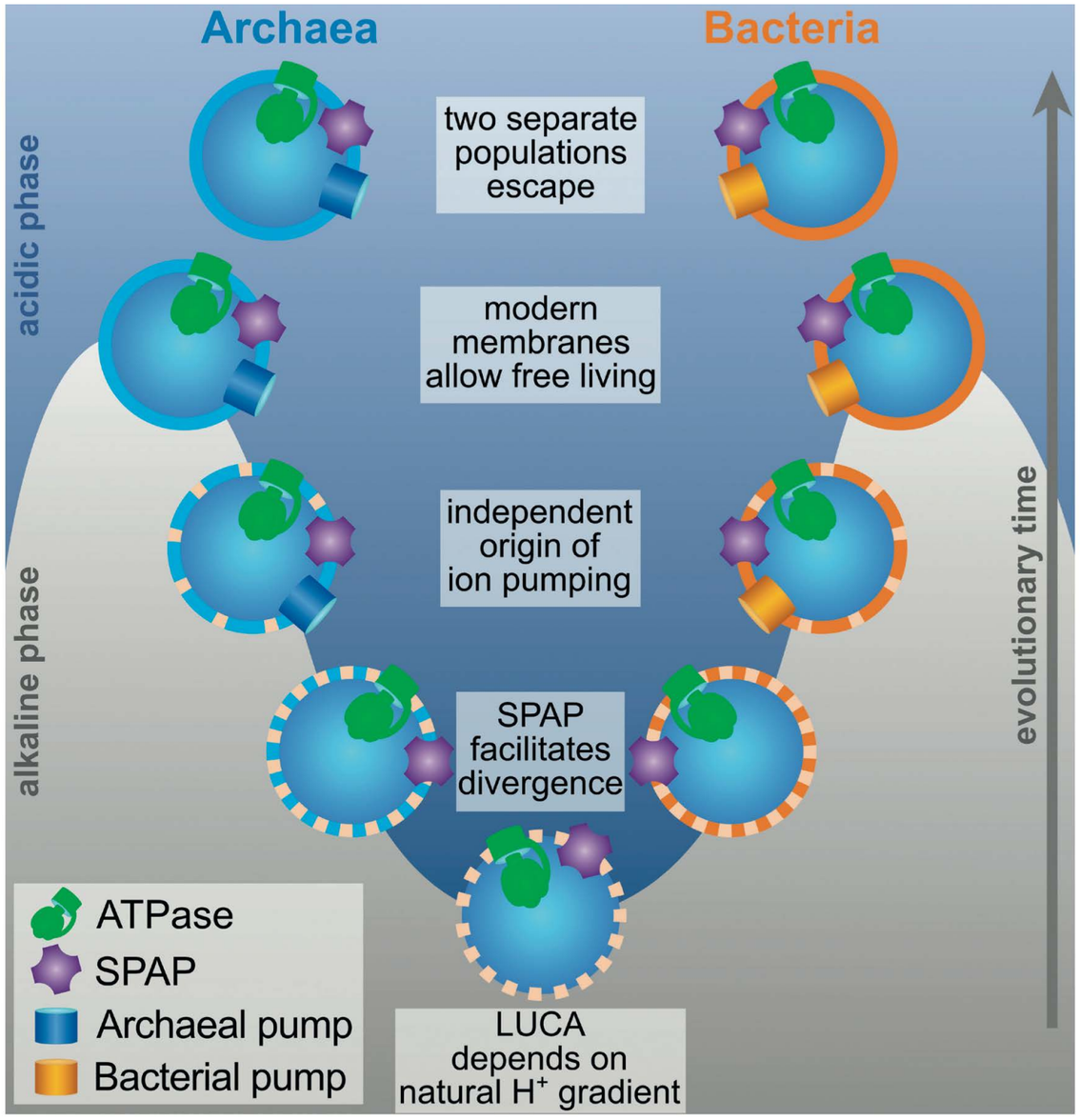


Figure 2. Independent escapes of bacteria and archaea from dependence on natural proton gradients in alkaline hydrothermal vents. Cells can only survive on natural proton gradients if they have leaky membranes, but active pumping across leaky membranes is futile. The evolution of a sodium/proton antiporter (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).

otic cells are at least 15 000-fold larger than prokaryotes, with genomes to match[17].
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 works[4]. 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 chloroplasts[18]. 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 30×106 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 Epulopiscium having as many as 200 000 copies of its complete genome. As Bill Martin and I showed[17], 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 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 molecules[13]. 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 archaeon[5].

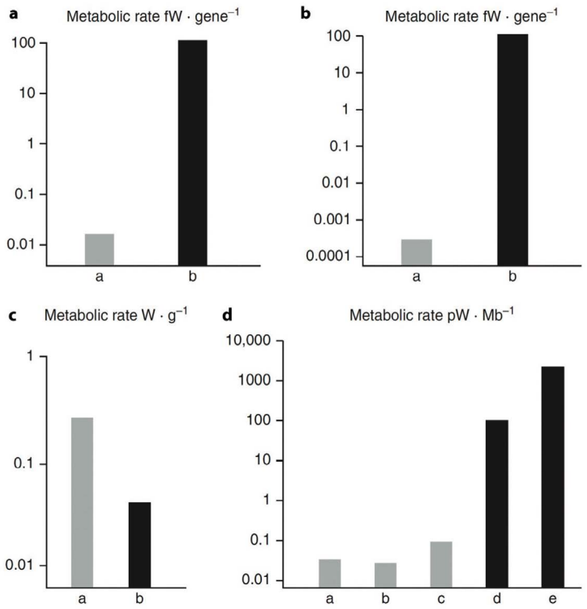


Figure 3. (a) Mean metabolic rate per gene in bacteria (a, grey bar) compared with eukaryotes (b, black bar), when equalized for genome size. (b) Data equalized for cell volume (15 000-fold larger in eukaryotes) as well as genome size. A single eukaryotic cell has ~100 000-fold more energy per gene than 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.

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 wall[4], 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 cell[4]. The balance between their requirements can explain unanticipated trade-offs between aerobic fitness and fertility, sexual maturation and lifespan[20]. 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 cells[21]. I hope these studies will make predictions that can be tested in the laboratory.

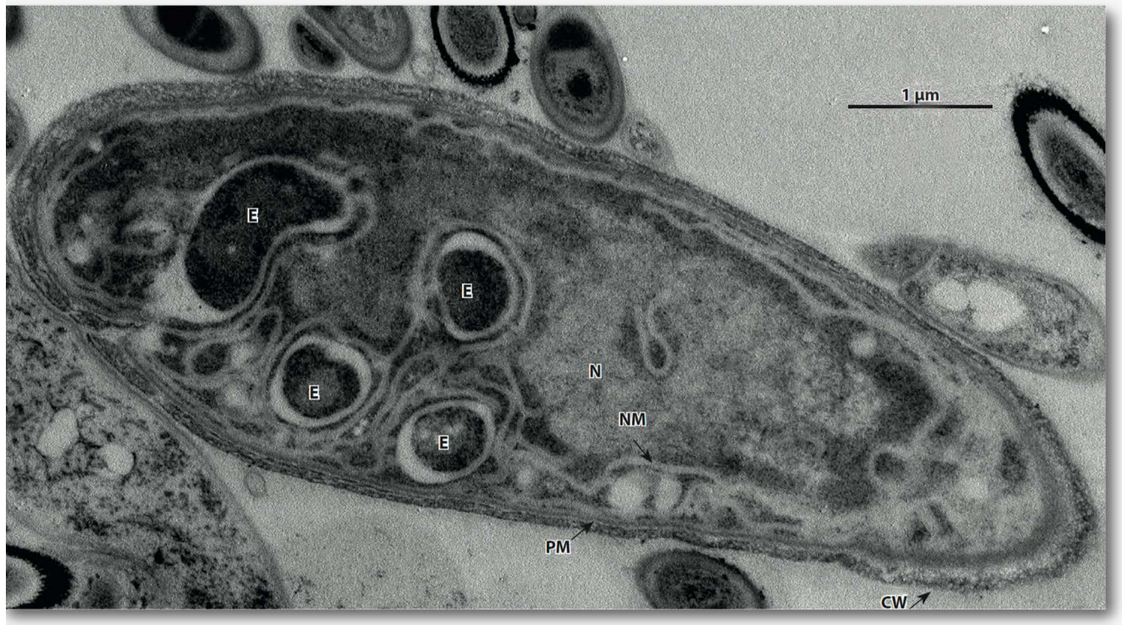


Figure 4. Prokaryote or eukaryote? This cell looks superficially like a eukaryote with a cell wall (CW), plasma membrane (PM) and nucleus (N) surrounded by a nuclear membrane (NM). It has several endosymbionts (E) that look like hydrogenosomes. It's quite big (10 µm in length), and the nucleus is large, 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.

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 Japan[22] (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. B **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 Sherringham, 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., Duhr, 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. Hadjivasiliou, 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

Southern Blot is published 3 times a year, summer, autumn and winter.

Editor: Astra Heywood.

If you have any meetings to promote, or articles, opinions or other ideas of what you would like to see published here, please email: astra.heywood@otago.ac.nz