Presenter: Professor Trevor Lithgow, Monash University - *2015*

Title: Germs within our cells: how bacteria evolve to power the human body. (15:10)

Time	*Dialogue*
00:08 00:47	So in preparing for this talk I was advised that the best way to pitch the talk was as if we were answering questions from someone at a party. So what do you do? I always used to tell people I worked in a record shop as it was way too hard to explain what it is I do do as a scientist. But here goes. So at Monash University as a scientist I work on bacteria and I work on imaging the outside surfaces of bacteria to have a look at the exists that they build into these surfaces in order to enable them to be able to secrete toxic proteins out and do damage. And this the bacteria do in order to conquer a new environment which is a problem if the new environment is us, one of our tissues such as our blood, our kidneys or our livers. What we do here in my research team at Monash University is look for these exit doors on the surface of bacteria with a view that we can design new drugs which would either close them over or stop them from being built in the first place.
01:09 01:33	And this matters a lot especially these days where we have the rise of anti-biotic resistant superbugs, bacteria that are becoming more and more resistant to the known drugs that we have where new drug based strategies are urgently needed as a major priority for human health. So we work on these bacteria, we look for these exit doors and for the last few years here at Monash University we've been surveying the surfaces using new technology such as the ones listed here that have become available really just in the last few years. With these new technologies we now have unprecedented detail of the surface of bacteria and you can see that apart from how dangerous they are really beautiful creatures. What I wanted to do though in this talk was to explain to you how it was that we came to work on these research questions because the inspiration for doing this really came out of left field.
02:05 02:30	Arh … This is the way that a lot of science works. We call it serendipity and it happened to us while we were working on something completely different, something called Mitochondria. I thought you might like a glimpse into how these sorts of journeys occur in science. So Mitochondria are the power houses that drive human cells. Mitochondria are found in human cells. They are found in the cells of all animals apart from humans, in addition they are found in all plants and in many, many single celled organisms found on earth. What they are not found in are bacteria and that's because the Mitochondria within our cells evolved from bacteria. What you can see here these long snake like strings are human protein molecules which travel into the Mitochondria. Dock into Mitochondria structures and thereby enslave the Mitochondria to work for the human cell.
03:00 03:39	As a result of this the Mitochondria now serve as part of the integrated circuitry within human cells and their primary responsibility is to make sure they burn the sugars and the fat molecules that we get from our diet to provide the best possible access to energy that we need to live. This idea, this concept that Mitochondria evolved from bacteria comes from an idea that was first raised in the early part of last century. It was quite controversial and it only really started to get traction in the eighties and the nineties. In 1995 I came back from Switzerland and set up a lab here in Australia to work on protein import into Mitochondria and to try and understand this overall process. We had some success, as did a number of labs around the world but there was always one enigma stuck in what was otherwise a really beautiful piece of human biology and that was the ancestral bacteria that gave rise to Mitochondria had on their surfaces doors that would let proteins out, but they never had doors on their surfaces that let proteins in because bacteria don't do that.

Time	*Dialogue*
04:09 04:37	And so we started to ponder while we were thinking about the evolution of Mitochondria this idea that it was that evolution might have come up with a solution to this problem to derive ways in which molecules could to come in through the out door. So in setting our experiments we were mindful of words from Francois Jacob (1920-2013), who was a great thinker and won a Nobel prize in 1965and continued even after then with big ideas with big ideas provoking the growing field of molecular biology in terms of the role in genes and the way in which genetic mechanisms drive evolution. I really love this particular quote from Jacob where he is making a comparison between the process of evolution and the hobbies of a tinker.
04:56 05:24	So it's this idea that perhaps molecules inside cells are available as if they were rusty parts cast aside at the back of someone's shed where they can be repurposed at an appropriate time if there is some reason to do so. And you can see here he says "It might well be of some use." For what? That depends on the opportunities." This sort of foresight was really remarkable because at the time that Jacob was throwing these ideas around in the middle 70's there was no way of knowing that the principles of evolution really held true at a molecular level. He was speculating about what might be true, based on the ideas of Darwin and others since then that were known to be true at the macro level. So with experiments that we've done and also experiments done by many people around the world on all sorts of biological systems it became evident that that in fact the Darwinian principles do hold true all the way down to the molecular level.
05:51 06:12	So we wanted to do experiments and we wanted to understand the process of evolution of how bacteria became mitochondria and how it was that protein export doors could be converted to protein import doors. To do those sorts of experiments, we are experimentalists. To do those sorts of experiments you need an experimental system and that means having a model organism, something that you can work with in the laboratory. Now we know about the family tree of mitochondria. We know where they came from because we can trace back using genome sequence information the ancestry of mitochondria and we know that they arose from a group of bacteria that are referred to as alphaproteobacteria. There are plenty of alphaproteobacteria that still exist on the earth today, free living as well as bacteria that live inside cells.
06:35 07:05	And in looking through these the people in my lab realised that colibacta was the perfect experimental model. It was perfect because for decades bacterial geneticists had been developing the tools that were needed to manipulate the genes of colibacta to understand the molecular components of the colibacta cell and indeed to be able to image the colibacta in action, live colibacta cells and look at individual molecules as they went about their business doing what bacterial cells do. So this was a great experimental system there and ready to be used for the sorts of things that we wanted to address. Someone that I have never met is one of the world's best colibacta biology experts working at Yale University in the US and after some Skype chats and some e-mail discussions with her we realised that colibacta really was the perfect system for us to do the sorts of experiments that we wanted to do in addressing this big question in evolution.
07:31 07:50	And I want to make this very clear that in science you can phone a friend. So in fact you can phone a perfect stranger. If you have a good idea and you know that someone that is working on something that could be useful to you, even if they are on the other side of the world, even if you have never met them before, you can get together with them. You can discuss the sorts of experiments that you would like to do and if they make sense to both of you then you end up doing experiments that neither of you could both do possibly alone. And this sort of International collaboration is a real bedrock of the way in which we do science these days.

Time	*Dialogue*
08:03 08:31	So back to our story. So in looking through colibacta we were looking for something that might have been ancestral to these mitochondrial import doors. Now we know what those doors look like because we have seen then in mitochondria. We use imaging technology to know exactly what they look like. One of the key ones that we know about is something that has the mysterious name of TIM 23. Tim 23 operates as a well-oiled machine. It's kind of like a powered revolving door for bringing proteins into the mitochondria and we know exactly what it looks like in molecular detail. A really talented post-doctoral fellow in my lab decided to go and have a look at colibacta to see if she could see something that looked like TIM 23. Indeed she did but what she saw was actually much more remarkable than we thought and it started to makes us think back to these words of Jacob.
08:56 09:21	So what she was able to see was that in colibacta there are all of the molecular components that you need to make a TIM 23, machine, a doorway but in colibacta they don't hang together as a well-oiled machine but rather they operate independently. Each of them doing a much simple job in complete ignorance of what the others are doing. So the process of evolution then was not about creating some new protein components but it was rather about taking protein components that were already existant in the bacteria and cobbling them together in to a new arrangement so they could fulfil a new function. And so to paraphrase Jacob, they really were of importance for what? … for this particular opportunity.
09:44 10:12	So this we thought was remarkable and we wrote it up and sent off our paper describing our findings to the top journal in biology. They kept it for six weeks of review and then the editors returned back to us a very short reply saying they would not publish the paper because the ideas behind it were too controversial. Now I of course got my dander up on this and tried to argue with them exactly what does too controversial mean and indeed what is so wrong about controversial ideas in science? But the thing about scientific publishing is the editors hold all the cards and once they have made a decision unless there is actually proof positive that there is something completely untoward has been done, you don't get your way and in this case we didn't. So the paper wasn't published in that particular magazine.
10:33 10:53	Not to be stopped we went ahead and contacted the National Academy of Sciences and asked them to publish the work in their journal PNAS (Proceedings of the National Acadamy of Science) . They kept is for several weeks of appropriate review and then the editor got back to us and said yes … but. One of the peer reviewers had decided that he or she, and you never know with anonymous peer review. That's one of the beauties. He or she had an idea that there was one more experiment that we should do and the experiment should give a sense of exactly how hard it was for the bacterial proteins to evolve to become the mitochondrial machine. This was a huge challenge. What the reviewer was in fact asking was for us to recapitulate in a test tube evolutionary history.
11:21 11:50	It was a huge challenge but on the other hand it was a really important point and we agreed that if this could be done experimentally it would add greatly to the paper and a graduate student in the lab decided to take the challenge on. So he took one of these bacterial proteins, called TIMB and he mapped out all of the mutations that could be possibly made and prioritised them just based on what we know about TIMB and the mitochondrial TIM23 and started on a set of experiments where he would gradually go through this list making mutation after mutation to get a sense of how many would be needed. It matters a lot too because the sceptics of evolution would tell you that it takes thousands of mutations to do something like this and that's they would say is the argument why evolution doesn't make senses and doesn't explain biology.

Time	*Dialogue*
12:12 12:41	The experiment said something different. It took one mutation. So the experimental system was set up in such a way that we took away one component of the mitochondrial TIM23 doorway, this machine, we put back into the system the TIMB protein from a bacteria and that's shown here in blue, (the TIMB molecule is in blue) and the rest of this golden machine the TIM23 is shown in gold. Now when you do that the cells will die because the TIMB protein from bacteria can't properly engage with the machine. It's the wrong part in a sense. It can't engage with the machine so the protein import process doesn't work and since making mitochondria is an essential part of living then cells that have this condition set up in them will die. But it only took one change to change this small red bit to the bit now shown in green and the TIMB protein did engage with the mitochondrial TIM23 machine.
13:08 13:37	Mitochondrial protein import reved up and it keeps going for ever, so you can restore this this function and you can convert this simple bacteria protein to a component of the mitochondrial TIM23 machine simply by this one small change. We sent the paper back to PNAS and they said immediately … yes this is ready for publication. Now I was on holidays in Dubrovnik (Croatia) when the paper came out in press and I remember getting an e-mail from my eldest son who said "Dad your work is all over the internet" and it turned out that this paper had been picked up by Richard Dawkins' website – 'A clear thinking Oasis' they had editorialised it and they rated it as a must read paper and that kicked off a whole fury of discussion from people who were interested in cell biology and evolution, both professional scientists as well as non-professionals, the general public and also from people who were sceptical about evolution.
14:02 14:22 14:56	And there was several months then of really heated discussion and I think really productive discussion about what this really means in general terms. And that made it all worthwhile and it really all came together then. We had taken a really tough problem and we had set up the experiments that could be done in order to solve the problem and come up with a solution that was worth putting out there in public. We had in so doing learned ways, new ways and adapted new ways to visualise these protein doors, these indoors and outdoors and many of the people in my lab along the way of this had started to use these new technologies to go back and look at some pretty awful bacteria and the way that they used their export doors in order to cause human disease. And I can now stop pretending that I work in a record shop. Thank-you.