



The mitochondria mystery

In the 1990s, French scientists wanted to see what happened to a mouse brain when they messed with the creature's mitochondria, the structures that generate energy inside most complex cells. The team looked at two mouse strains, called H and N, that carry slightly different mitochondrial-DNA sequences.

It was clear that the H mice learned to navigate mazes faster than their N cousins, but when the team swapped the mitochondria — creating H mice with N mitochondria and N mice with H mitochondria — their performance changed. Mitochondria from N seemed to slow down the learning process for H mice. N mice, meanwhile, improved slightly with H mitochondria¹. And

the team, led by geneticist Pierre Roubertoux at INSERM, the French National Institute for Health and Medical Research in Marseilles, found other changes in behaviour, and in brain anatomy, too.

The results came as a surprise, because such differences between mitochondrial genomes were seen as neutral — having no biological effect. “The long-held view was that the genetic variation we find within the mitochondrial genome doesn't affect function,” says Damian Dowling, an evolutionary biologist at Monash University in Melbourne, Australia.

That view has been changing. A growing body of evidence suggests that mitochondria do not just produce energy, but also influence a wide range of cellular processes, from cell death to immune responses, and

The ‘powerhouses’ of the cell may have more roles than expected. Could that generate problems for mitochondrial replacement therapies?

By Garry Hamilton

that variations in the organelle matter very much. Variants in mitochondrial DNA are now linked to many common human conditions, including neurodegenerative diseases, cancer and ageing.

The effects of these variants may come about through the organelle's long-evolved partnership with the much-larger nuclear genome. Studies in a handful of organisms have shown that just as for H and N mice, swapping healthy mitochondria between closely related strains can cause a mismatch between the genomes and can change important traits. The evidence, say Dowling and others, should raise questions about the safety

of a procedure that will soon be used in humans.

In February, the UK government approved mitochondrial replacement therapy, a technique that would allow a woman with a mitochondrial disorder to give birth to healthy children by pairing her nuclear DNA with the healthy mitochondria from a donor's egg. The approval came after a 3.5-year effort to review the safety and ethics of creating individuals with DNA from three people (what some refer to as three-parent babies). And although many scientists lauded the decision, some worry that it is premature. “They're not looking at the bigger picture,” says Ted Morrow, an evolutionary biologist at the University of Sussex in Brighton, UK, who is arguing for more-rigorous safety testing.

ILLUSTRATION BY SÉBASTIEN THIBAUT

A common refrain in favour of the therapy is that the genetic contribution from mitochondria is very small. And against the 3 billion base pairs of DNA and 20,000 genes found in the human nucleus, the mitochondrial genome can seem pretty insignificant (see 'A complicated relationship'). Inherited solely through a mother's egg, it comprises fewer than 17,000 base pairs and just 37 genes. But one cell can have thousands of copies of the mitochondrial genome, compared with just two of the nuclear genome — one from mum and one from dad.

Mitochondrial DNA also accumulates mutations incredibly fast, at about ten times the rate of nuclear DNA — and geneticists can use the resulting variation as a sort of molecular clock. The clock has allowed scientists to create a human family tree that shows several broadly related mitochondrial genomes, known as haplogroups, emerging in Africa somewhere around 150,000 years ago, including two that gave rise to the thousands of smaller haplogroups now found around the world.

The standing view was that the genetic differences between mitochondria in these groups were little more than a reflection of past migrations. But during the 1980s, researchers began to challenge that assumption. "Mitochondria control a central component of metabolism," says David Rand, an evolutionary biologist at Brown University in Providence, Rhode Island. "So it followed that this variation ought to be very interesting."

One way to examine whether mitochondria in one population work differently from those in another is to swap them. Such experiments would be unethical in people and impractical in many other animals, so Rand turned to fruit flies. He cross-bred two fly strains with different mitochondria and then repeatedly back-crossed them until the mitochondria from one were neatly paired with the nucleus of the other.

He then put fruit flies with similar nuclear genomes but different mitochondria together in a cage, and found that flies with specific mitochondrial genomes would quickly come to dominate the population². Something in the mitochondria was giving them a survival advantage. Subsequent work by Rand, Dowling and others has shown that it is not just the mitochondrial genome, but rather its interaction with the nuclear one that seems to be affecting a range of traits, including lifespan, reproductive success, rate of development, ageing, growth, movement, morphology and behaviour.

The findings extended beyond inbred laboratory animals such as fruit flies and mice. Over the past two decades, Ron Burton at the Scripps Institution of Oceanography in La Jolla, California, has found that cross-breeding closely related populations of tiny crustaceans known as copepods from tide pools on the Pacific coast often leads to a massive fitness breakdown for the animals³. Two clues led Burton to suspect that the reason was a mismatch between nuclear and mitochondrial DNA. First, the populations had very different mitochondrial genomes. Second, energy production was at the heart of all the sickly organisms' deficiencies.

The clincher came when Burton chose females from the unhealthy animals and mated them with males from the same population as the females' mothers. The resulting offspring, which once again had a natural combination of mitochondrial and nuclear genomes, were healthy. "That's pretty striking," says Burton. "And we did it with multiple different crosses."

Extending these results to mammals has been difficult: Roubertoux's mitochondrially mismatched mouse lines took more than 20 generations and 12 years to develop. But there are a few studies that have found similar results. Douglas Wallace, who heads the Center for Mitochondrial and Epigenomic Medicine at the Children's Hospital of Philadelphia, combined the nucleus from a lab-mouse strain with mitochondria from a mouse known to contain two different, but normal, mitochondrial genomes. His group found that the modified mice

had altered circadian rhythms — the natural oscillations that follow a roughly 24-hour cycle — performed worse in mazes and seemed more stressed in certain experimental conditions, compared with unmodified animals⁴.

In humans, there is only indirect evidence that the common variation found in the mitochondrial genomes of healthy individuals could have biological effects. Certain mitochondrial haplotypes have been linked to disorders such as type-2 diabetes, Parkinson's disease and cancer, and normal variation in the mitochondria is thought to influence general physical traits such as longevity and elite athleticism⁵.

"Correlations are just correlations," says Göran Arnqvist, an evolutionary biologist at Uppsala University in Sweden, "but there's now a large enough number of them to in itself provide ample evidence that there's something going on with mitochondrial DNA".

Powerhouse pairing

The question remains exactly how these variations could affect such a broad range of biological functions. Part of the answer seems to lie in their ties with the nuclear genome. Roughly 1,500 nuclear genes are

involved in mitochondrial function, including around 76 that encode proteins which bind to mitochondrially derived peptides.

Common variants could alter how these proteins interact. If a mitochondrially derived protein needs to fit snugly against a nuclear counterpart, even tiny changes in one partner could disrupt that binding, a possibility supported by 3D modelling^{6,7}.

A study published in 2009 compared mitochondria from two common human European lineages, called haplogroups J and H, in cells with the same nuclear DNA⁸. It showed that cells with haplogroup J mitochondria contained more than twice as many copies of mitochondrial DNA as those with haplogroup H, a difference that would be expected to have a big influence on the production of

mitochondrial proteins.

Such effects could alter the rate at which mitochondria supply energy, with consequences for many cellular activities. But emerging evidence points to other ways that mitochondria could have broad biological implications.

Various molecules created during the production of energy, such as free radicals, may have a direct influence on processes involved in ageing, inflammation and in some basic cell functions. And in May, a team of researchers led by Gerald Shadel at Yale University in New Haven, Connecticut, showed in mice that mitochondrial DNA can itself trigger an innate immune response against viral infection⁹. "They're not just power factories," says Rand. "They're also in a sense a nerve centre, a thermostat for the cell and how it's doing."

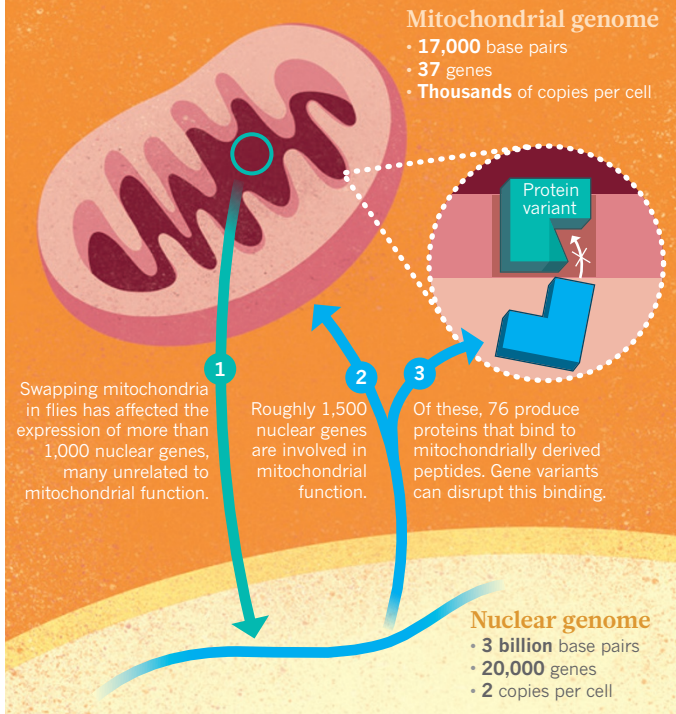
Researchers have also found evidence for a new class of mitochondrially derived peptide that might be encoded by sequences in other mitochondrial genes. One of these is humanin, a small peptide discovered by Japanese researchers in 2001 that increases sensitivity to insulin in diabetes-prone rats and mice¹⁰. The gene that encodes it is thought to reside in the mitochondrial gene for 16S ribosomal RNA. In March, researchers in the United States found a second potential example, MOTS-c, which is encoded by a small stretch of DNA tucked away in another gene. MOTS-c functions like a hormone, and when injected into mice helps to enhance insulin sensitivity and protect against obesity¹¹.

Some researchers now suspect that mitochondrial DNA produces a vast array of biologically active molecules — other small peptides as well as short stretches of RNA — that are part of a network of cross-communication between the mitochondrial and nuclear genomes. "The very viability of complex life — eukaryote life — depends on a really coordinated,

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A complicated relationship

The mitochondrial genome has evolved in concert with the nucleus of complex cells for hundreds of millions of years. Evidence suggests that even slight disruption of that relationship could have unexpected effects.



intimate set of interactions between these two genomes,” Dowling says. It is a partnership that has shaped and been shaped by aeons of evolution.

Given how well evolution has tuned this communication, many biologists are concerned about disrupting it in mitochondrial replacement therapy. The results of mitochondria-swapping experiments in other organisms, they say, should not be overlooked. “We haven’t seen anything fundamentally different between flies and humans in terms of interactions between the mitochondria and the nucleus,” says Klaus Reinhardt, an evolutionary biologist at the University of Tübingen in Germany.

The health effects may not be dramatic, says Burton, and they might not become apparent until decades after birth. “But I think there’s a definite possibility that you’d see things like disrupted fertility function, various forms of metabolic syndromes and changes in things that relate to metabolism in general.”

Call for caution

Reinhardt, Dowling and Morrow outlined their concerns in a 2013 paper¹² in *Science*. They called for studies aimed at addressing how mammals born after mitochondrial replacement fare in adulthood, and argued that scientists should at least look into haplotype matching — ensuring that the mitochondria from the donor and recipient come from the same haplogroup before transplant. Moving ahead at this juncture, they argued, “would place an experimental risk on families.”

But other researchers disagree. Scientists at Newcastle University, UK, and at Oregon Health & Science University (OHSU) in Beaverton, two institutions that pioneered mitochondrial replacement therapies, pointed to perfectly healthy macaque monkeys born at OHSU in 2009 after the procedure¹³.

They also pointed out that most of the evidence for risk stems from studies that used strains of flies and mice that had been highly inbred — a process that would increase the genetic differences between the strains and therefore produce a greater ‘mismatch’ when the mitochondria are swapped. They argued that such studies have little relevance for

human populations that interbreed all the time. The “lack of any reliable evidence of mitochondrial–nuclear interaction as a cause of disease in human outbred populations,” they wrote, “provides the necessary reassurance to proceed”.

Doug Turnbull, who heads the Newcastle group, also argues that correlations between different human mitochondrial haplotypes and common diseases are not definitive. “If we’re struggling to find a signal,” he says, “is that really something that’s likely to cause major difficulties?”

Ultimately, government approval hinged on a 2014 report prepared by a scientific review panel set up by the Human Fertilisation and Embryology Authority (HFEA), the body that regulates assisted-reproduction treatments in the United Kingdom. The panel’s chair, Andy Greenfield of the Medical Research Council, would not comment for this story, but the HFEA provided a written response to questions. It stated that deliberations were “time-consuming and as complex as the data themselves”, adding that most respondents presenting evidence to the panel viewed these issues as “at best minor or non-existent”. In its final report, the panel recommended that haplogroup matching be considered “as a precautionary step”. But it also stated that the benefits of doing so are “likely to be minimal”.

Some of the critics of the decision grant that mitochondrial replacement may be worth the risks for women who want to avoid passing rare and devastating disorders on to their children. Many, however, think that more time is needed to assess the risks. There is also concern that proponents of the therapy trivialized the role of mitochondria — particularly by likening mitochondrial replacement to changing the batteries in a camera. Critics argue that a failure to appreciate all the other processes in which the organelle is involved could lead to inadequate controls and wider application of mitochondrial replacement in fertility clinics.

“You may have a few thousand people who suffer from mitochondrial diseases,” says David Keefe, a reproductive biologist at New York University’s Langone Medical Center. “There are tens of millions of women who have infertility who may see this as a way to have the batteries charged in their eggs.”

At least one clinic in the United States has used cytoplasm from donor eggs to ‘normalize’ the eggs of women being treated for infertility, starting in the late 1990s (see *Nature* 509, 414–417; 2014). The procedure, which probably transferred mitochondria as well, resulted in 17 births before the US Food and Drug Administration requested safety studies and the clinic stopped offering the procedure in 2001. Little is known about the health of the children born as a result of the procedure.

Turnbull rejects the slippery-slope argument. “In the UK, the legislation is very clear that mitochondrial donation can only be used to prevent serious mitochondrial disease,” he says. “I do not think there is any good evidence it would be useful for anything else.”

Although no one knows what the rapidly growing field of mitochondrial research will uncover next, both sides agree that there is no way to say for sure what will happen when doctors swap mitochondria in humans, short of actually doing it. For Dowling, at least, it is one scientific debate that he would rather not win. “I’d like to see this work so female sufferers of mitochondrial disease can have unaffected children,” he says. “So I hope we’re wrong.” ■ SEE EDITORIAL P.425

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1. Roubertoux, P. L. *et al. Nature Genet.* **35**, 65–69 (2003).
2. Hutter, C. M. & Rand, D. M. *Genetics* **140**, 537–548 (1995).
3. Ellison, C. K. & Burton, R. S. *Evolution* **62**, 631–638 (2008).
4. Sharpley, M. S. *et al. Cell* **151**, 333–343 (2012).
5. Hudson, G., Gomez-Duran, A., Wilson, I. J. & Chinnery, P. F. *PLoS Genet.* **10**, e1004369 (2014).
6. Osada, N. & Akashi, H. *Mol. Biol. Evol.* **29**, 337–346 (2012).
7. da Fonseca, R. R., Johnson, W. E., O’Brien, S. J., Ramos, M. J. & Antunes, A. *BMC Genomics* **9**, 119 (2008).
8. Suissa, S. *et al. PLoS Genet.* **5**, e1000474 (2009).
9. West, A. P. *et al. Nature* **520**, 553–557 (2015).
10. Muzumdar, R. H. *et al. PLoS ONE* **4**, e6334 (2009).
11. Lee, C. *et al. Cell Metab.* **21**, 443–454 (2015).
12. Reinhardt, K., Dowling, D. K. & Morrow, E. H. *Science* **341**, 1345–1346 (2013).
13. Chinnery, P. F. *et al. PLoS Genet.* **10**, e1004315 (2014).