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The Inside Story of Mitochondria
The fascinating organelle is biology's playground

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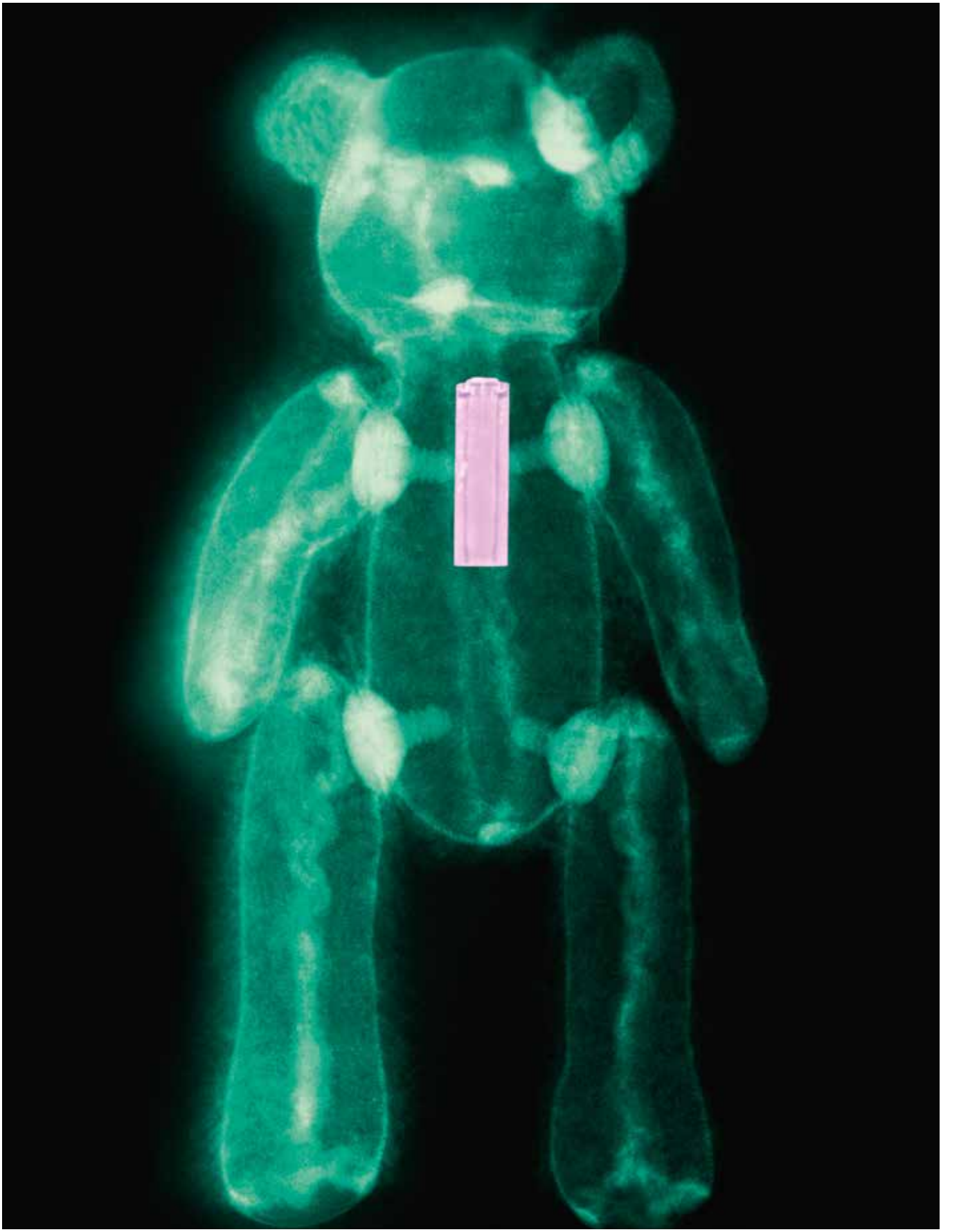

The main task of mitochondria is to generate energy for cells, but the organelles are also involved in processes like cell death or autophagy.

THE **INSIDE** STORY OF **MITOCHONDRIA**

By Sarah Williams

Studying the diversity of mitochondria in living organisms allows researchers to weave a tale of the organelles' past. Their evolution is an intriguing story by itself, but knowledge about how they function also has many implications for our health.

Around a billion years ago, a cell captured a nearby bacterium, enveloping it completely. Once inside, the bacterium became a survival advantage, helping the cell to generate energy from oxygen. The cell divided, and within it the bacterium divided as well, and that happened again and again, and a whole host of cells were born, all containing these little helpers. Over the eons, though, the new organelle – today known as the mitochondrion – lost some of its genes entirely. In some organisms, mitochondria grew so large that they almost took over the cells' interiors. In others, mitochondria shrunk and nearly disappeared – or, in at least one case, disappeared entirely. And in all cases, mitochondria – with their own distinct DNA from the rest of the cell – evolved different ways of doing things than the rest of the cell.

"The mitochondrion is molecular biology's playground," says Michael Gray of Dalhousie University in Canada. "Even though the function of mitochondria is pretty well conserved throughout evolution, the way in which the genes are arranged and expressed is very diverse. As long as you can get those key proteins churned out, you can do it in all sorts of ways."

In March 2017, Gray was one of about 60 researchers who gathered in southern Germany for Boehringer Ingelheim Fonds' 115th International Titisee Conference to discuss the evolution and diversity of mitochondria. For four days, biochemists, geneticists, and physiologists shared perspectives on what has driven some mitochondria to be such outliers, and how the organelles are linked to human health.

"We are witnessing a renaissance in mitochondrial research," says Vamsi Mootha of Harvard Medical School in Boston, USA, chair of the conference. "Human studies are underscoring the importance of mitochondrial dysfunction in a number of different conditions, and advances in genome sequencing are pointing to the remarkable diversity of mitochondria across the tree of life. For these reasons, it was a terrific opportunity to convene this meeting."

Over the past few decades, researchers have been trying to piece together the evolutionary history of mitochondria and have also uncovered more than 275 disease-causing mutations in the mitochondrial DNA of humans. The severity of the diseases and breadth of symptoms underscore the importance of the organelle – people with mitochondrial diseases can have seizures, muscle weakness, exercise intolerance, developmental delays, and breathing problems, just to name a few symptoms.

Most biology textbooks describe mitochondria with the same catchphrase: they are the "powerhouses of the cell." It is an appropriate analogy, since in nearly all eukaryotic cells, oxidative →

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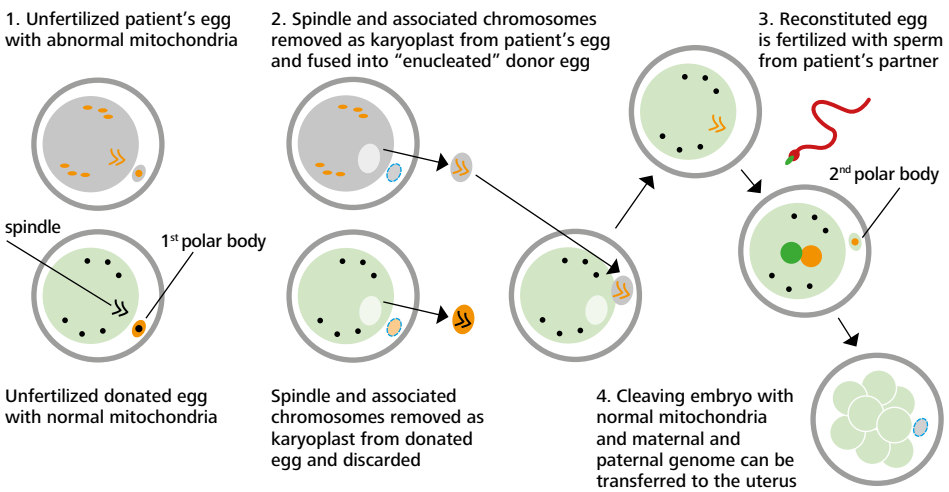
SOMETIMES IT TAKES THREE – HOW WOMEN WITH MITOCHONDRIAL DISEASE CAN HAVE HEALTHY CHILDREN



Source: Maternal spindle transfer Bredenoord, A and P. Braude (2010) "Ethics of mitochondrial gene replacement: from bench to bedside" BMJ 341.

Mitochondrial replacement therapy uses healthy donor mitochondria to replace the mother's abnormal ones.

In 2015, the United Kingdom became the first country in the world to pass laws allowing the creation of human embryos from three people: a mother, father, and mitochondrial donor. The technique – called mitochondrial replacement therapy (MRT) or mitochondrial transfer – allows women with mitochondrial diseases to have children without passing on their defective mitochondrial genes. In MRT, as with all forms of *in vitro* fertilization, eggs are harvested from a woman, sperm collected from a man, and fertilization occurs in a lab dish before an embryo is implanted back into the mother's uterus. But with MRT, eggs are also collected from a female donor with healthy mitochondria. Mitochondria from the donor eggs replace the mitochondria in the mother's eggs or in the fertilized embryo, depending on the exact technique used. To date, a small handful of babies have been born using MRT and dubbed "three-person babies" in the popular press. From a purely genetic standpoint this is correct, as they carry genes from three different people. However, the proportions differ widely, with only a tiny fraction of one per cent – the genes encoded in the mitochondria – coming from the mitochondrial donor.

phosphorylation – the process by which cells convert oxygen and nutrients to the cellular energy currency ATP – occurs inside mitochondria. But it also glosses over the many other functions of the organelles. They house metabolic pathways, export iron-sulphur clusters to modify proteins, and are involved in cell death, autophagy, and other processes such as stress adaptation, immune response, and cell proliferation.

In animals, mitochondria are usually passed to offspring from their mothers; paternal mitochondria are currently thought to be degraded inside sperm or shortly after fertilization. There are rare exceptions. Mollusks, for instance, inherit mitochondria from both parents, and in some insects paternal inheritance of mitochondrial DNA has been reported. The usual inheritance pattern, though, means that maternal lineages can be traced using mitochondrial DNA. By studying the small genetic differences in people's mitochondria, for instance, scientists have calculated that all women today descended from a single woman – dubbed the Mitochondrial Eve – who lived around 200,000 years ago in East Africa.

Many scientists, though, are focused on events even further in the past than the Mitochondrial Eve and are interested in the origin of the mitochondrion itself. Half a century ago this year, in 1967, evolutionary biologist Lynn Margulis, then at Boston University, proposed that mitochondria arose from a bacterium engulfed by an ancient cell. The general idea had been around since at least since 1883, but her paper is now considered a landmark in the modern theory of endosymbiosis. Since then, genetic sequencing has revealed that mitochondria share a number of DNA sequences with α-proteobacteria, a class of bacteria that the organelles most likely originated from. But the details of the endosymbiosis that led to mitochondria are hard to uncover.

"There are lots of α-proteobacteria out there and we don't really know what the α-proteobacteria that became the mitochondria was like," says Gray. "We're inferring from current organisms what might have happened a billion or so years ago."

To make these inferences, Gray has focused on the mitochondria of protists – the most diverse group of eukaryotes. His group was the first to describe the mitochondrial genome of *Reclinomonas americana,* a freshwater protozoan that contains a larger mitochondrial genome (96 genes) than most organisms; human mitochondria, for instance, contain 37 genes, just 13 of which encode proteins. This large collection of genes, Gray's group has

shown, represents a more ancestral state of the organelle. In fact, the protist's mitochondria still possess RNA polymerases resembling those found in bacteria, not animals. The finding was considered one of the final and most convincing pieces of evidence to support the endosymbiosis theory of mitochondrial evolution.

Since characterizing the *R. americana* mitochondrial genome, Gray and his colleagues have sequenced the mitochondrial genomes of other related protists – called jakobids – and found an even larger set of mitochondrial genes in *Andalucia godoyi*. But even these jakobids share only a small handful of their mitochondrial proteins – 10 or 20 at the most – with α-proteobacteria. So what happened to the rest of the bacterial genes? When did the mitochondria lose them, and where else did they acquire genes?

"We have some general answers about how that might have happened," says Gray. "But also a whole series of questions." For instance, there is still debate as to whether mitochondria arose at the same time as the eukaryotic cell. One hypothesis, for example, states that mitochondria and the larger amount of energy they provided were a prerequisite for true eukaryotic cells to arise. An argument in favour of this theory is that eukaryotes arose only once. However, other scientists have suggested that cells gained eukaryotic traits by other means before later acquiring mitochondria, which made them more efficient.

To explain how mitochondria acquired genes that do not resemble α-proteobacteria, Gray proposed what has been dubbed the pre-endosymbiotic hypothesis. It says that another bacteria may have joined with an *Archaea* cell before mitochondria arose to provide some extra energy to help the eukaryotic transition. Later, genes from that other bacteria may have ended up in mitochondria as well. But he admits that it is hard to come by convincing evidence. If the entire story of mitochondria cannot be worked out by comparing them to bacteria, perhaps homing in on the diversity of individual pathways within the mitochondria of organisms can help.

Anastasios Tsaousis of the University of Kent, UK, studies the synthesis of iron-sulphur clusters by the mitochondria of microbial parasites. The ensembles of iron and sulphur are necessary, for example, for the functioning of proteins involved in metabolism and oxidation – both inside and outside mitochondria. The production of these clusters is considered one of the most critical functions of mitochondria; it cannot be done by other organelles.

Most mitochondria generate iron–sulphur clusters using one pathway – called the ISC machinery. But *Blastocystis*, a type of single-celled parasite, also has a sulphur mobilization (SUF) system usually found only in plants and *Archaea*. When *Blastocystis* cells are depleted of oxygen, they switch on the SUF system to synthesize the clusters. Studying how the ISC machinery and SUF system compare, and how and when they each evolved in mitochondrial history, Tsousis says, may teach us more about some of the broader questions relating to mitochondria's past.

Even the energy-generating components of the mitochondria responsible for their powerhouse moniker vary in some organisms. Since the mitochondrial respiratory chain depends on oxygen as an electron acceptor, organisms that live in low oxygen environments often have evolved alternative methods of generating ATP, such as special oxidases. Kiyoshi Kita of the University of Tokyo and Nagasaki University in Japan studies parasites – including those that cause malaria and African sleeping sickness in humans – that can use the chemical fumarate as an electron acceptor. Thus, they can generate energy in places where oxygen is scarce – including the human gut. "This ability to adapt to low oxygen by using fumarate is not only seen in parasites, but also in cancer cells," says Kita. "So it's a good target for chemotherapy." Kita has already begun to screen chemical compounds to identify potential drugs to block the fumarate pathway in the mitochondria. Other researchers are testing in human cell lines whether and how different mitochondrial respiratory chain enzymes from other organisms can help to treat genetic deficiencies in human mitochondria.

While the SUF and fumarate pathways set some mitochondria apart, the details of how some mitochondria package and process their DNA and RNA get researchers to exclaim "strange" and "bizarre". After all, in the nuclei of organisms from amoebas to humans, there is little variation in the mechanisms used to replicate or translate genes, so any deviance is surprising. →

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All known polymerases lengthen a strand of nucleotides, such as DNA or RNA, by adding to their 3' end. Jackman and her collaborators, however, have found a polymerase in the mitochondria of slime mold that moves in the opposite direction: it adds a histidine to the 5' end of a mitochondrial tRNA molecule. Since discovering this so-called Thg1 enzyme, Jackman has identified members of the Thg1 superfamily in eukaryotes, bacteria, and archaea.

Studying how these mitochondrial enzymes work is not only interesting from an evolutionary and enzymatic perspective, says Jackman, but from a clinical one as well – mutations in the *THG1L* gene in humans have been linked to delays in development.

Julius Lukes of the University of South Bohemia in the Czech Republic finds the massive, oddly packaged mitochondrial genomes of protists very intriguing.

"The story always goes that humans must have more complexity in every way than protists that are a millimetre long," says Lukes. "Curiously, though, protists have more complex and bizarre mitochondria than humans."

Lukes' research revolves around euglenozoan protists, a large and diverse group of organisms that all have a single large mitochondrion – unlike most eukaryotic cells containing many small mitochondria. And while the mitochondrial DNA of most organisms is organized in a relatively simple circle or line of genes, the DNA of euglenozoans is, in general, much more complex. In some cases, it is simply large; in other cases, it is packaged in a way that is unheard of anywhere else – in thousands of tiny, interlocked kinetochore discs.

The best-studied euglenozoans are trypanosomes, which include parasites such as those causing sleeping sickness and Chagas disease in humans. Since their mitochondria are so different from humans, they allow drugs targeting their respiratory enzymes to be highly selective. Trypanosomes and related euglenozoans have exceptionally massive mitochondria – both in terms of the amount of DNA and the size of the organelle, with the mitochondria taking up 80 or 90 per cent of the entire interior in some cells. "It's like the mitochondria aren't just part of the cell, they are actually taking over the cell," says Lukes. And the massive mitochondrial genomes, Lukes has discovered are not big because they encode lots of proteins; rather, their RNA is edited down radically post-transcriptionally, resulting in a similar number of mRNAs or proteins. In some parasites that Lukes has studied, genes – whether contained in the nuclear or mitochondrial DNA – involved in processes in the nucleus, cytoplasm, Golgi apparatus, and endoplasmic reticulum are reduced in number and complexity, while genes involved in mitochondrial processes remain complex. It is as if the cell is shifting more complexity – and perhaps more function and importance – to the mitochondria, Lukes says.

"Molecular biologists tend to see an advantage in everything in biology, and think that everything in a cell must be there with a purpose," he says. In this regard, mitochondria seem to provide us with a particular intriguing snapshot of evolution in progress, he adds. This appears to be confirmed by researchers led by Vladimir Hampl of Charles University in Prague, who recently reported the existence of a protozoan with no mitochondria at all. However, how they evolved is not known.

Of course, most of the mitochondria's functions are not unnecessary; on the contrary, small mutations to mitochondrial genes can cause severe disease phenotypes in humans. The diseases can be difficult to diagnose and the same mutations may cause different symptoms in different people, even affecting different tissues in their bodies. In most cases, treatments are currently limited to lifestyle and dietary changes – such as ketogenic diets, supplemental calories, or anaerobic exercise – to ease symptoms and boost the number of healthy mitochondria in cells.

However, as basic research on mitochondria progresses – whether it aims to uncover their evolutionary history, the enzymology of surprising mitochondrial proteins, or how mitochondria vary between species – we may better understand these diseases and obtain clues toward targeted treatments.