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POWERHOUSE OF DISEASE

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Many of the genes affecting mitochondria — tiny energy suppliers of cells — reside in the cell nucleus. Nick Lane joins the hunt for these sequences that may underpin diseases such as diabetes.

Some of Gerald Shulman's patients at Yale University School of Medicine are young and slim. There's little wrong with them, and probably won't be for a decade or two. Yet tests raise an ominous spectre. All are the children of parents with type 2 diabetes, and, already, in their twenties, they are becoming resistant to insulin, the hormone that should be keeping their blood sugar levels under control.

The problem seems to lie in their muscles, whose cells lack tiny lozenge-shaped structures called mitochondria. These normally function as powerhouses inside cells, burning up fuel with oxygen. Long regarded as the cell's menial coal-shovellers, mitochondria are emerging as key players in health and disease. The 'organelles' are unusual in having their own DNA, although many of the genes that once resided in the mitochondria have, over evolutionary time, decamped to the cell's nucleus. Shulman is one of a number of scientists who think that tracking down the hundreds of 'missing' genes that have shifted to the nucleus is going to change the way we think about common diseases such as diabetes and Parkinson's.

Mitochondria store the energy released from food in the form of a molecule called ATP, which is used to power virtually all forms of work in the body, from muscle contraction to protein synthesis. Your body's mitochondria generate an impressive total of some 65 kg of ATP every day. The double-membraned organelles (see picture, overleaf) perform this feat thanks to a process called chemiosmosis, which pumps protons across one of their membranes. ATP is generated when the current of electrically charged protons, produced by this pump, passes through tiny protein motors embedded in the same membrane.

Ancient union

As well as looking like them and using chemiosmosis in the same way as bacteria, mitochondria contain a bacteria-like genome. Indeed, mitochondria were once free-living bacteria; they were engulfed by larger cells two billion years ago in a unique merger that gave rise to all complex, or eukaryotic, cells. The size of the genome housed within the mitochondrion varies between species. All mammals, for example, have retained just 37 genes, whereas yeasts have retained between 40 and 50, and some plants as many as 100.

But mitochondrial genomes did not start out so small — they probably once contained at least a few thousand genes, inherited from the free-living ancestor of mitochondria[1]. Exactly what happened to most of these genes is a moot point, but the evolution of a stable symbiotic relationship within eukaryotic cells led to hundreds, perhaps even thousands, being simply transferred to the cell's main genome in its nucleus. These transfers meant that mitochondria became dependent on the host cell for virtually all their functions. Today, some 99% of human mitochondrial proteins are encoded in the nucleus; all the proteins and other molecules required to build mitochondria are synthesized in the main body of the cell, then imported into the organelle. Only a fraction of these genes has been identified; the rest lie hidden in the vast code of the nucleus's genome.

This enigmatic 99% is now the focus of intense scrutiny. There are good reasons to believe that genes affecting the mitochondria could play a central role in human health and disease. Most of the genes that have remained in the mitochondrion have been linked to a series of devastating diseases, indicating the importance of fully functional mitochondria to human health.

Genes residing in the mitochondria pose a particular problem, however — in part because

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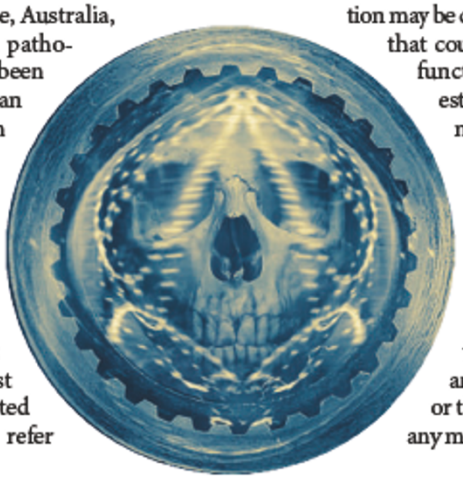
they are unusually prone to damage. Unlike nuclear genes, which are wrapped in protective proteins and stored safely away in the nucleus, mitochondrial genes are vulnerable to attack from highly reactive molecules called free radicals; these are generated during energy production. In mammals, the mutation rate of mitochondrial genes is 10 to 20 times higher than that of the nuclear genes.

The idea that mutations in mitochondrial DNA could cause metabolic diseases, or even ageing, has gained credence since Fred Sanger's group at the University of Cambridge, UK, sequenced the human mitochondrial genome[2] in 1981. According to David Thorburn, at the Murdoch Children's Research Institute in Melbourne, Australia, in the decades since, pathogenic mutations have been discovered in more than 30 of the 37 human mitochondrial genes. These alterations range from changes to single DNA bases to deletions of large sections of the genome. Their effects are a long list of rare disorders, best diagnosed and treated by specialists, who refer to themselves as mitochondriacs.



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The most common childhood condition is Leigh syndrome. This affects about 1 in 40,000 children and tends to develop within the first year of life, often after a viral infection. In most cases, degeneration of the central nervous system leads to loss of muscular coordination and death within a few years, although some children survive into their teens. Lethal infantile mitochondrial disease is much rarer but even more deadly. Children born after an uneventful pregnancy tend to have seizures soon after birth, make few or no spontaneous movements, and die of respiratory failure within weeks. Other conditions have relatively mild symptoms. A common feature of all these diseases is that they tend to worsen with age. Indeed, it is the cumulative effects of free-radical attacks, and the corresponding build up of mitochondrial mutations that may underpin aging.

Faulty engine

Mitochondria, along with their tiny genomes, are normally inherited only from the mother — they are present in huge numbers in the egg, whereas the handful in sperm is marked up for destruction in the fertilized egg. This gives at least some mitochondrial diseases a maternal-inheritance pattern. Even so, trying to spot mitochondrial diseases by looking to the mother can be grossly misleading, and has downplayed the importance of these organelles in disease. More than 80% of diseases known to be linked to faulty mitochondria don't follow a maternal-inheritance pattern at all.

Why not? At least partly because some mitochondrial diseases may be caused by mutations in the nuclear genes encoding mitochondrial proteins. So far, mutations in more than 30 nuclear genes have been shown to give rise to mitochondrial disease. Thorburn, however, estimates that as much as a tenth of the population may be carrying genetic disorders that could affect mitochondrial function[3]. This is based on estimates of the number of mitochondrial genes in the nuclear genome and the incidence of recessive genetic disorders. He echoes a favourite catchphrase of mitochondriacs: "Mitochondrial deficiency can theoretically give rise to any symptom, in any organ or tissue, at any age, and with any mode of inheritance."

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The actual contribution of nuclear genes to mitochondrial diseases is highly uncertain for a simple reason — we are surprisingly ignorant of what the nuclear genes actually are, and how they interact with mitochondrial genes. In mammalian mitochondria, the best guess is that the nuclear genome encodes 1,500 distinct mitochondrial proteins. So far, barely half have been formally identified, and of these, the function of a sizeable proportion remains unknown.

Nonetheless, the evidence that mitochondrial proteins are responsible for a lot more mischief than once thought is growing. A series of inherited conditions not thought of as 'mitochondrial' have turned out to be caused by mutations in genes encoding mitochondrial proteins[4]. For instance, Friedreich's ataxia (a progressive loss of coordination of voluntary movements) is caused by mutations in a gene encoding a small mitochondrial protein called frataxin. Hereditary spastic paraplegia (a progressive weakness and stiffness of the legs) can be caused by mutations in a mitochondrial enzyme, paraplegin.

Other, more complex degenerative conditions, such as Parkinson's disease, progressive-blindness diseases and other nervous-system conditions also involve mutations in mitochondrial proteins[4]. Even cancer can be caused by mutations in nuclear genes encoding mitochondrial proteins[5]. Examples are now cropping up almost every year, and together they are beginning to focus attention on the central role of mitochondria in disease.

These examples have all unexpectedly turned out to be 'mitochondrial', after years of tracking down candidate genes for the diseases. But new tools are letting scientists turn the old approach on its head. Rather than starting with an inherited condition and trying to track down the genes responsible, researchers are starting off with the mitochondria themselves, and attempting to hunt down the proteins needed to build them. Tracking down this array of proteins, or the mitochondrial 'proteome' is no easy task; researchers rely on a combination of methods to build an accurate picture, including mass spectrometry to identify proteins and molecular-biology techniques to measure RNA, the molecule used by cells as a template from which to build proteins.

All the techniques based on this bottom-up approach have strengths and weaknesses, but by taking the best information from each, scientists are gradually piecing the mitochondrial proteome together. Once the normal proteins have been identified, any oddities in patients can be

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pinpointed. The abnormal protein can be mapped on to the candidate genes for disease, and any causal mutations involved identified.

In 2003, Vamsi Mootha, a computational biologist at the Broad Institute in Cambridge, Massachusetts, and his colleagues published a list of several hundred new mammalian mitochondrial proteins[6], raising the known mammalian total to around 600. Crucially, however, Mootha's group also examined tissue variations. In mice, they found that around half the mitochondrial proteins identified were present in four different tissues — brain, heart, liver and kidney. But the other half tended to be tissue-specific, with some degree of overlap (around 50%) between different tissues.

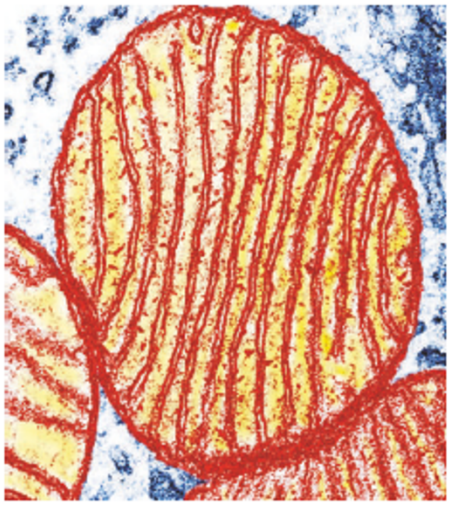
Building a powerhouse

Mitochondria are well known to carry out specific tasks in different tissues; for example, they make haem, part of the oxygen-carrying protein haemoglobin, in bone marrow cells. But the finding that hundreds of mitochondrial proteins varied in amounts from tissue to tissue came as a shock. If corroborated, this variation suggests that the control of mitochondrial gene activity is very sophisticated. And this has a corresponding impact on our susceptibility to disease; the more complicated the control system, the more likely it is to fail.

Mootha's group reported the first two tissue-specific mitochondrial proteins, known as Errα and Gabpa/b, in 2004 (ref. 7). Both control gene activity, which in turn affects how much mitochondria replicate themselves in particular tissues. If the expression of Errα and Gabpa/b is high, then mitochondria replicate at a high rate, and become densely packed in the tissue. If their expression is lower, the number of mitochondria and their ability to burn fuel falls. Critically, Errα and Gabpa/b influence mitochondrial function and density in particular tissues, notably the heart and muscle, and play a lesser role in tissues such as the liver. Mootha notes that this tissue specificity makes them valuable drug targets, because it restricts the potential for side effects in other tissues.

The next question for Mootha and his team was what happens if the activity of Errα and Gabpa/b falls? They predicted that a fall in the number and capabilities of mitochondria in particular tissues would result— a finding that Mootha and others had previously reported in the muscles of patients with diabetes. Sure enough, Mootha's lab found that the activity of these proteins was lower in the muscles of patients with type 2 diabetes[8]. But could such a change be a root cause of diabetes, or was this merely a consequence of some other metabolic problem, such as obesity?

Type 2 diabetes has two cardinal features.



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Genes in the nucleus that encode proteins for the mitochondria (above) could underpin diseases.

The first is that cells become resistant to the effects of insulin, the hormone made by the pancreas that normally prompts them to take up and burn glucose. The second is high levels of glucose in the blood, or hyperglycaemia. Insulin resistance is typically one of the earliest signs of diabetes, often preceding hyperglycaemia by decades.

Faulty mitochondria have already been linked to the second phase of the disease — namely the emergence of hyperglycaemia. Defective mitochondria in the pancreas fail to burn sufficient glucose, so the levels of ATP in pancreatic cells are abnormally low. But these cells rely on ATP levels to help them estimate the amount of glucose in the blood. As a result, the cells do not sense glucose properly, do not release appropriate amounts of insulin and the blood glucose level creeps up[9].

> "Faulty mitochondria may well be the cause of diabetes, but we still don't know what makes them faulty."

But what about insulin resistance? Shulman thinks that faulty muscle mitochondria could underlie insulin resistance in muscle tissue and was intrigued by Mootha's findings. "We've been working with volunteers who have a high genetic risk but a low 'lifestyle' risk of diabetes. We hope to eliminate confounding factors such as obesity, or indeed the early stages of diabetes itself, and focus on the earliest underlying genetic influences."

Complex pathways

Shulman's group has found three striking oddities in the muscle cells of the young volunteers: they are often very insulin resistant, taking up about 60% less glucose in response to insulin compared with the muscle cells of unaffected people; they have a low mitochondrial density, about 40% lower than normal; and they have a large accumulation of fat molecules, or lipids, around 60% above normal[10].

The key, says Shulman, is the high level of lipids. Lipids can cause insulin resistance by jamming the cellular machinery that helps receive the hormone's signal. But what causes their levels to rise in the cell? There are two main possibilities: a faster rate of lipid breakdown and delivery to muscles from fat tissues; or a defect in the muscle mitochondria themselves. If faulty mitochondria don't burn fats as fast as they should, then that could lead to a build-up of lipids inside the muscle cells. That would suggest the primary genetic cause of type 2 diabetes lies in the mitochondria. Faulty mitochondria also contribute to obesity, by not burning fats properly, and obesity in itself exacerbates diabetes.

Shulman's group could find no evidence that abnormal fat breakdown and delivery from fat tissues was responsible, and so turned to look at possible faults in mitochondria.

Following up on Mootha's findings, the team looked as whether a mutation in the genes controlling the tissue-specific mitochondrial proteins Errα and Gabpa/b could underpin the low density of mitochondria in the volunteers. The result, published in December last year, was a surprise. They could find no such mutations, implying that the reduction in gene expression measured by Mootha was not the primary cause of diabetes. The primary fault must lie in another, as yet unknown pathway governing mitochondrial proliferation and activity.

So faulty mitochondria may well be the cause of diabetes, but we still don't know what makes them faulty. Yet with hundreds of unknown mitochondrial proteins still to uncover, Shulman and Mootha have a long list of possible suspects to work through. Whether they will get results in time to help Shulman's young volunteers is an open question, but the answers seem set to revolutionize our understanding of disease. ■

Nick Lane is a science writer based in London.

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