
CELLULAR ALARM

Paul Fisher looks to a slime mould to find that mitochondrial disease is caused by the signalling activity of a cellular alarm protein.

There's nothing more irritating! It's the first day of a long-anticipated holiday and I am already salivating in anticipation at the aromas, sights and sounds of breakfast cooking – a mess of ham and eggs, toast and “real” coffee. There's nothing better than a leisurely breakfast with my wife on the deck overlooking our garden, I muse. An instant later my reverie is shattered: that bloody smoke alarm! There's nothing more irritating!

I leap into action, and in the minute or so it takes to retrieve the offending device and perform the emergency operation to remove its battery, I have already plotted several different and gruesome ends for its inventor. Now I can relax. Ah, the tranquillity!

But imagine if the infernal thing did more than make that infernal, piercing noise? What if it turned off the electricity and gas supply to the house (for the best possible safety reasons) and closed and securely locked every door and window (to restrict the oxygen supply to the fire and to limit its spread)? And what if I couldn't turn it off – ever? My smoke alarm, intended to provide me with early warning of a looming fire danger, will have itself caused a chronic malady worse than the crisis it was intended to prevent.

Recent research in my laboratory using the cellular slime mould *Dictyostelium discoideum* shows that this may be precisely what happens in an eclectic and poorly understood group of genetic disorders known as mitochondrial diseases. The mitochondria are the powerhouses of our body's cells, and are responsible for converting the energy in our food into a useful chemical form for our cells.

Bacterial Ancestry

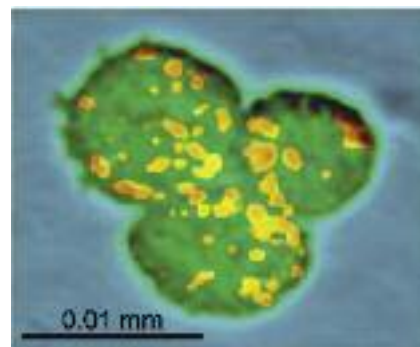
The mitochondria we see today in the cells of modern organisms are distant descendants of bacterial invaders that took up residence a few billion years ago inside the ancient cells from which we are all descended, humans and slime moulds alike. In the aeons that followed, the mitochondria and their host became dependent upon one another – the mitochondria provided energy for the host but placed almost all of their genes in the care of the host by transferring them to the host's chromosomes.

There are now only a small number of protein-coding genes left in the mitochondria – 13 in the case of humans and 33 in *Dictyostelium*. The proteins they encode are synthesised and used inside the mitochondria, and mutations that affect them can cause mitochondrial disease. The remainder of the couple of thousand proteins required in the mitochondria are encoded by genes in the host nucleus. Once synthesised by the host cell, these proteins must be imported into the mitochondria to carry out their intended functions.

Mitochondrial diseases can therefore arise from mutations both in mitochondrial genes and in nuclear genes encoding mitochondrial proteins.

Mitochondrial Diseases

In humans, mitochondrial diseases result in a confusing variety of clinical outcomes that can include blindness, deafness, epilepsy, stroke-like episodes, exercise intolerance, inability to coordinate limb movements, heart disease, diabetes, Parkinson's disease and kidney disease. Furthermore, the pathological consequences of mitochondrial disease are bewilderingly different in every patient – the same genetic mutation can



A group of three *Dictyostelium* amoebae (green) with stained mitochondria (gold).

Original photos by A.U. Ahmed

cause completely different symptoms in different individuals, while different mutations can produce very similar clinical outcomes. This results from the complicated nature of human development, which superimposes upon the underlying cell biology a range of individual, tissue and age-related differences that can obscure regularities in the disease process. These overlaid complexities have restricted our ability to understand the underlying disease process in mitochondrial disorders.

By studying mitochondrial disease in a simple creature, *Dictyostelium* (Dicty to its friends), we have avoided the confusing complications imposed by animal and human development. Dicty is a soil microorganism and is known as a social amoeba because of its ability, when faced with starvation, to embark upon a survival program where up to a million solitary cells aggregate together to form a multicellular migratory organism called a “slug”. Only 1–2 mm in length, the slug migrates to the surface of the soil where it forms a fruiting body composed of a droplet of spores sitting atop a stalk and a basal disc.

Dicty slugs find the surface by following light (a behaviour called phototaxis) and temperature gradients (thermotaxis) that exist between the surface and the subsurface regions of the soil. As a PhD student I became interested in this as a model for how cells sense and respond to external stimuli, and began to conduct genetic studies aimed at finding out how it works. It was this interest that ulti-



Electron microscope photographs of multicellular stages in the development of *Dictyostelium discoideum* from the early aggregate through the mound, slug and Mexican hat to the final fruiting body. The inset shows merged phase contrast and fluorescence microscope images of single amoebae.

mately led to the new understanding of mitochondrial diseases we are now acquiring.

Genetic Studies

In 1990, at the International *Dictyostelium* Conference in Turin, Italy, research assistant Zofia Wilczynska and I reported the first Dicty mutants to have been created by randomly inserting a foreign DNA molecule into genes of interest. The idea was to disrupt genes this way, identify them as being of interest based on the abnormal behaviour of the mutants and then use the inserted foreign DNA as a molecular tag to help isolate and identify the genes.

It turned out to be not that simple. We created a phototaxis mutant this way but it was several frustrating years before we were able to overcome the technical difficulties of isolating and identifying the gene we had disrupted.

Imagine our shock and surprise when we finally did so and it turned out to have nothing directly to do with phototaxis, but instead to be a mitochondrial gene. We had inadvertently created mitochondrial disease in Dicty, and the primary symptom was deranged responses to light and temperature gradients!

It took a few more years for a postdoctoral fellow in the laboratory, Christian Barth, to prove that we were right before we felt sure enough of our findings to publish them in 1997. It had always been believed that mitochondrial diseases resulted from there being insufficient energy to support cellular functions. This paper provided the first hints that the disease pathology might arise not from energy insufficiency *per se* but from deranged sensing and processing of signals.

Our next step was to look for regularities in the outcomes of mitochondrial disease in Dicty to find out if they were always the same regardless of which gene was defective. Martha Kotsifas, a PhD student in my laboratory, went on to

create mitochondrial disease in Dicty by genetically inhibiting the function of a nuclear gene that encodes an essential mitochondrial protein called chaperonin 60. This protein is found only in bacteria, in mitochondria and in the chloroplasts of plant cells. Its function is to help other proteins fold properly into their final correct shape. Without it or with too little of it, many mitochondrial proteins can't fold properly and therefore can't function.

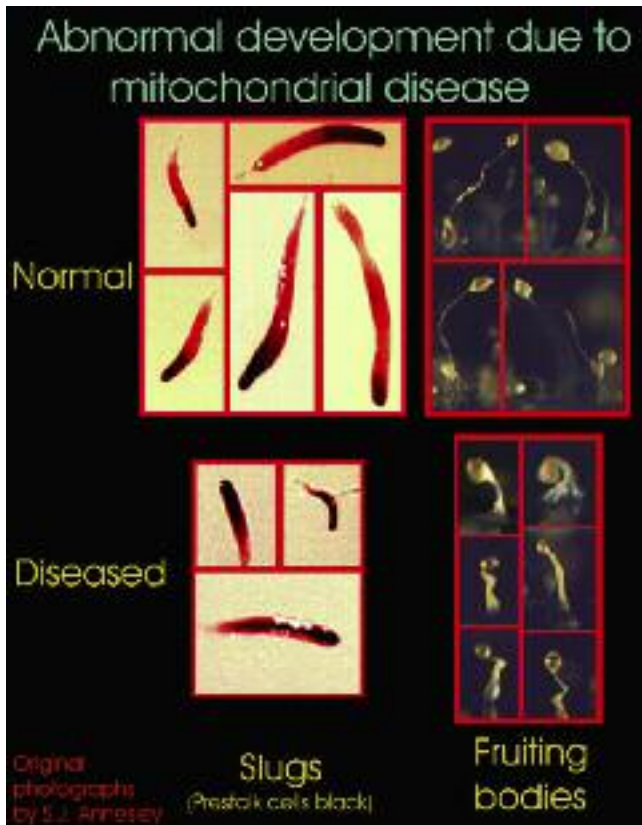
Martha found that genetically inhibiting the supply of chaperonin 60 protein to the mitochondria in Dicty produced a mitochondrial disease with the same outcomes as we previously observed when we disrupted a mitochondrial gene. Martha's results were published in 2002. Another PhD student, Lisa Francioni, has shown that it does not matter which gene in the mitochondrial genome is disrupted: the outcomes are also the same.

These regularities point to there being a common process underlying the pathology of mitochondrial disease, regardless of its genetic nature. It is the very simplicity of the Dicty life cycle that has allowed us to see this regularity, obscured as it is in humans by the complexities of mammalian development and ageing.

If mutations affecting different genes produce the same mitochondrial disease outcomes in cells, what might be the common underlying mechanism they all affect? Despite the bewildering array of clinical outcomes, it has been suggested that all of the known genetic causes of human mitochondrial disease should impair the capacity of the mitochondria to produce useable energy for the cell. This is true of Dicty as well. All of the disease-causing mutations we had introduced would have compromised the capacity of the mitochondria to produce energy for the cell.

What would happen if we created mutants in which some aspect of mitochondrial function is impaired that does not affect energy production? Together with Peter Beech's group at Deakin University and some US colleagues, we created mutants in which mitochondrial division is partially impaired so that the mitochondria in the cells divide less often. The result was that the mitochondria within the cells grew longer on average before dividing, but their capacity to produce energy was almost certainly unchanged. These mutants did not show the typical symptoms of mitochondrial disease in Dicty – for one thing, their phototaxis was normal.

However, phototaxis and thermotaxis are not the only things that go wrong in mitochondrially diseased Dicty. From the beginning it was clear that the severity of the genetic disease we created was different in every mutant and that in many cases growth and multicellular development were also deranged. The diseased cells grew more slowly regardless of whether they were fed bacteria (their natural food source) or a nutritious broth. During multicellular development they preferentially formed stalk cells rather than spores, so the fruiting



Normal and mitochondrially diseased *Dictyostelium* slugs and fruiting bodies. Fruiting bodies are natural colour and the slugs are stained to show the prestalk cells (which will later make the stalk in the fruiting body).

bodies they produced were abnormal with short, thick, misshapen stalks.

Yasuo Maeda's group at Tohoku University followed this up and showed that cells throughout mitochondrially diseased slugs had already embarked on the pathway that will lead later to stalk cell formation in the fruiting body. Normally only cells in the tip of the slug would do this, while the great bulk of the remainder would have started instead on the first steps towards spore formation.

What all of these processes have in common – phototaxis and thermotaxis, development, growth and proliferation – is that they consume energy and they are controlled by regulatory signalling pathways within the cell.

Signalling Disorder

Although compromised energy production is the underlying cause of mitochondrial disease, our results indicated that the first thing to go wrong in the cell as a consequence is its capacity to sense and respond to specific signals. This means there must be a link between energy metabolism and signalling processes. I began to search for such links.

One possibility was that cellular signalling mechanisms were simply more sensitive than other cellular processes to a shortage of energy. Another was that the mitochondria were fully integrated into the cell's signalling systems, so

that they were both listening and talking to the rest of the cell. Perhaps this conversation was being disrupted in mitochondrial disease.

Then, in 2001, I came across an article by Grahame Hardie of the University of Dundee about a protein called AMPK that is activated when energy supplies fall and is inhibited when they return to normal.

A cell needs the energy provided by the mitochondria for its movement, its growth and reproduction, its eating, its drinking, its listening and talking to other cells by means of chemical signals. If there is ever a shortfall in the energy available to carry out these activities, the cell needs to know and take appropriate remedial action. For example, without enough energy it would be disastrous for a cell to embark on a complicated and critical energy-consuming task such as replicating its chromosomes and undergoing division. The end for a cell that ran out of energy part way through this process would be every bit as gruesome as those I imagined for the inventor of the smoke alarm.

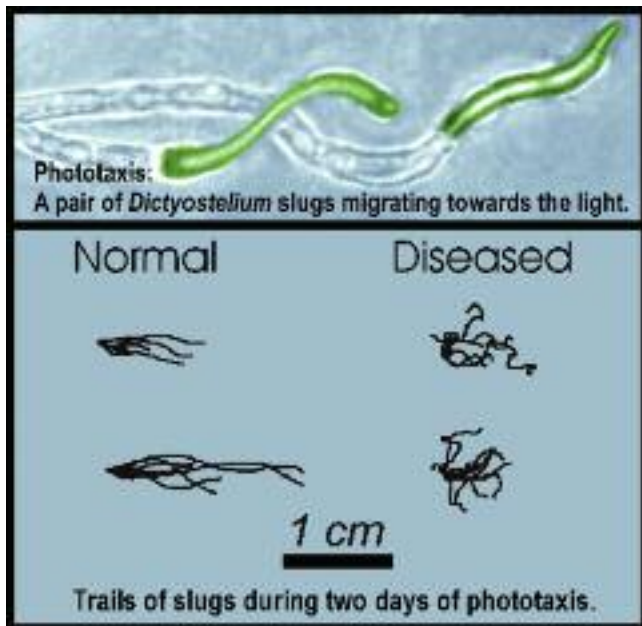
To avoid such a fate, the cell possesses its own smoke alarm – AMPK. Whereas my smoke alarm, when activated, merely sounds a warning (however irritating), AMPK takes immediate remedial action. It stimulates the production of more useable energy and shuts down a variety of energy-consuming processes, such as cell growth and division. These actions of AMPK bring cellular energy levels back to normal. At this point, life in a healthy cell can return to its usual rhythms.

In 2001, no one suspected that AMPK would play important roles in regulating pathways not directly related to energy production or conservation. However, AMPK was precisely the kind of protein I was looking for. It was a signalling protein itself, a protein kinase that worked by attaching phosphates to other proteins to control their activity, and it did so very sensitively in response to declining energy supplies in the cell. AMPK was at the crossroads between the cell's energy metabolism, its mitochondria and its signalling systems.

I decided to investigate whether inappropriate AMPK activation and signalling might be the cause of the pathological outcomes of mitochondrial disease. To do so I had to wait until the arrival in 2002 of Paul Bokko, a new PhD student from Nigeria, who was able to show over the next 4 years that AMPK, the cellular alarm protein, is indeed responsible for all of the defects caused by mitochondrial disease in *Dicty.* This work was recently published in *Molecular Biology of the Cell*, a high profile international scientific journal.

With the help of contributions from other students in the laboratory and some US colleagues, Paul obtained four lines of evidence for this conclusion:

1. Overproduction by the cell of an active form of AMPK mimicked mitochondrial disease, causing the same



Migration by healthy and mitochondrially diseased *Dictyostelium* slugs towards a light source on the right. Slugs are shown in false colour.

- defects – impaired phototaxis and thertotaxis, slow growth and deranged development with short, thick, misshapen stalks in the fruiting bodies.
- Genetic inhibition of the production of the AMPK protein prevented all of the deleterious outcomes in mitochondrially diseased cells. Phototaxis, thertotaxis, growth and development all returned to normal in spite of the mitochondrial deficiency.
 - Treatment of the cells with a drug that activates AMPK in mammalian cells caused a phototaxis defect similar to that caused by mitochondrial disease.
 - Nutrient uptake rates of both bacteria and broth were unaffected by mitochondrial disease because they are impervious to AMPK signalling and thus were also unaffected by genetically altering the AMPK levels.

Other experiments confirmed that, as in human cells, AMPK in Dicty stimulates the proliferation of mitochondria and the production of energy. By this means, chronic activation of the alarm protein AMPK in mitochondrially diseased cells can countervene the reduced energy-generating capacity of the mitochondria. Cellular energy levels can return to normal as long as, and because, AMPK is active.

As is mostly the case in cells from human patients with mitochondrial disorders, we found that energy levels were close to normal in our mitochondrially diseased Dicty strains. However, the ongoing activity of AMPK in mitochondrially diseased Dicty cells permanently impairs phototaxis, growth and development.

A Universal Alarm

We have shown that AMPK is responsible for the defects associated with mitochondrial disease in Dicty, but are there

grounds to believe this may also apply to humans? The answer is yes. In spite of the complexities of human mitochondrial disease, some things seem clear.

First, all of the mutations known to cause mitochondrial disease would compromise the capacity of the mitochondria to produce energy for the cell.

Second, cellular stresses that compromise mitochondrial energy production are known to activate AMPK in human cells.

Third, many of the cellular hallmarks of mitochondrial disease in humans are also known to be caused by activation of AMPK in human cells, including proliferation of mitochondria, impaired cell proliferation and growth, autophagy (a survival strategy in which the cell digests its own internal parts to recover energy) and apoptosis (a form of programmed cell death in which the cell suicides).

Fourth, both the mitochondria themselves and AMPK are found almost universally in living organisms (other than bacteria) because they serve universal functions that were also needed in the ancient cells from which we are all descended, humans and social amoebae alike.

Whether it is a healthy human or Dicty cell, AMPK activity can help solve a temporary energy crisis and bring energy supplies back to normal. Sadly, however, things will never be normal for a cell with a mitochondrial disease. Such a cell has a permanent genetic lesion that impairs the capacity of its mitochondria to produce useful energy. The powerhouses of the cell are running at less than normal capacity and AMPK, the cellular energy alarm, is chronically activated.

In a futile attempt to rectify the cell's energy problem, AMPK's remedial actions never cease – depending on the type of cell and the extent of AMPK activation it may fail to grow and proliferate, it may produce more mitochondria, it may fail to develop properly and it may undergo autophagy or apoptosis. It is these cellular abnormalities that result in the malfunction of the organs and tissues to which the affected cells belong, and that ultimately produce the pathological outcomes.

Tragically, the dire cellular consequences of AMPK action in mitochondrial disease may all be unnecessary. Like my smoke alarm, AMPK is fine-tuned to sense a pending crisis before the energy supplies become insufficient to support normal cellular activities. If only the cell could turn off the alarm, its life could be pretty normal.

This is a cause for hope. Although the cell cannot turn off AMPK, maybe we can. Maybe it will be possible to find drugs that will inhibit AMPK and maybe they will provide a way to treat these currently untreatable diseases. If so we will owe a lot to the simple social amoeba!

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