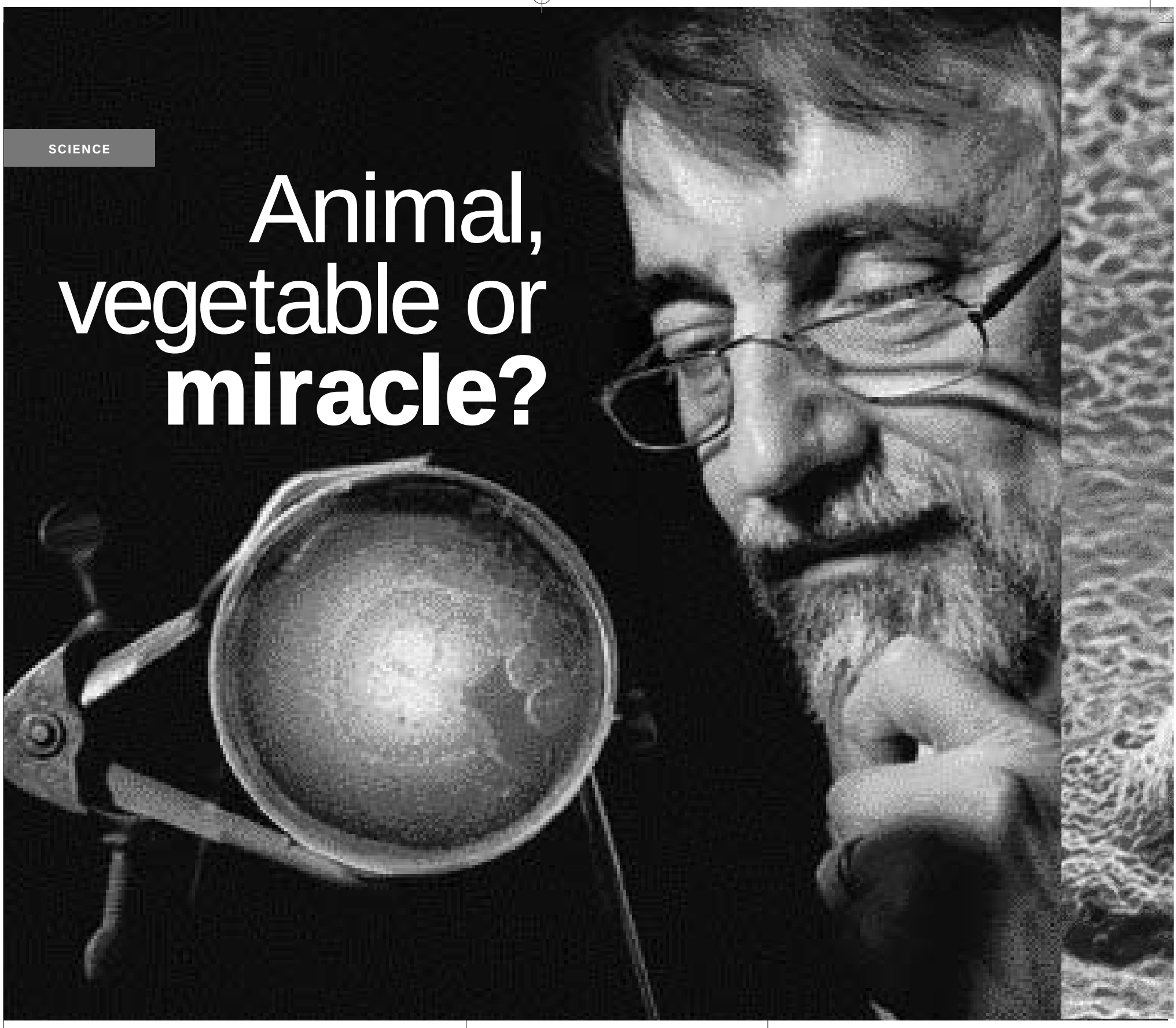
SCIENCE

Animal, vegetable or **miracle?**



It may be born in dirt but the humble slime mould is medicine's supermodel. Australian research suggests it could help us understand and cure a raft of diseases from Alzheimer's to epilepsy. Why then, asks **Katherine Wilson**, is no one funding its study?

If you're reading this in the suburbs, in the country, or near a city park, you're within gumboot-throwing distance of a slime mould community. "If you pick up a handful of soil in your garden," says Professor Paul Fisher, "you've probably got hundreds of thousands of them in your hand."

Sometimes they appear as yellow felt on the bottom of trees. Some look like fungi, some form slime on the soil surface, some incarnate as slippery slugs that wriggle above ground. One species forms a sludge that bears a likeness to vomit on the lawn, prompting dog owners to summon the vet. But if you suffer from some forms of Parkinson's, blindness, deafness, dementia, migraine, epilepsy, heart disease and diabetes – mitochondrial disease is the collective term for such illnesses – these slimy, mobile communities might hold the key to your wellbeing.

In 2000, to the astonishment of Japanese researchers, one slime mould species formed a worm-like sludge that plotted the shortest route through a complex maze to reach food at the other end. With no brain and no central nervous system, how, wondered scientists, could this fusion of cells know how to communicate, self-organise, specialise (some transforming into "leader" cells and others into "tail" cells), make decisions and mobilise? Could these cells possess an intelligence, of sorts?

Paul Fisher's life's work has been to solve mysteries such as these. A slim man in faded jeans and sneakers, he is calm, bearded and soft-spoken, with a cheerful warmth about him. Heading a team of 12 graduate scholars in the microbial cell biology laboratory at La Trobe University in Melbourne, Fisher is showing the world that, to an extent, humans are merely slime mould, and slime moulds are only human.

"We share around a third of our genetic material with the slime mould cell," Fisher explains. The very genes that encode instructions telling a slime mould cell to co-operate with its comrades, form a slug and slither about, are also those that influence some of our most debilitating diseases, such as cancer and dementia. And by mutating the slime mould's "co-operation" and "movement" genes, Fisher has found a way to cure many of our disease symptoms.

All but ignored in Australia, his discoveries have earned Fischer serious accolades internationally. Judith Armitage, professor of biochemistry at Oxford University, says Fisher's work forms "a foundation for understanding everything" about the causes and treatments of many genetic diseases.

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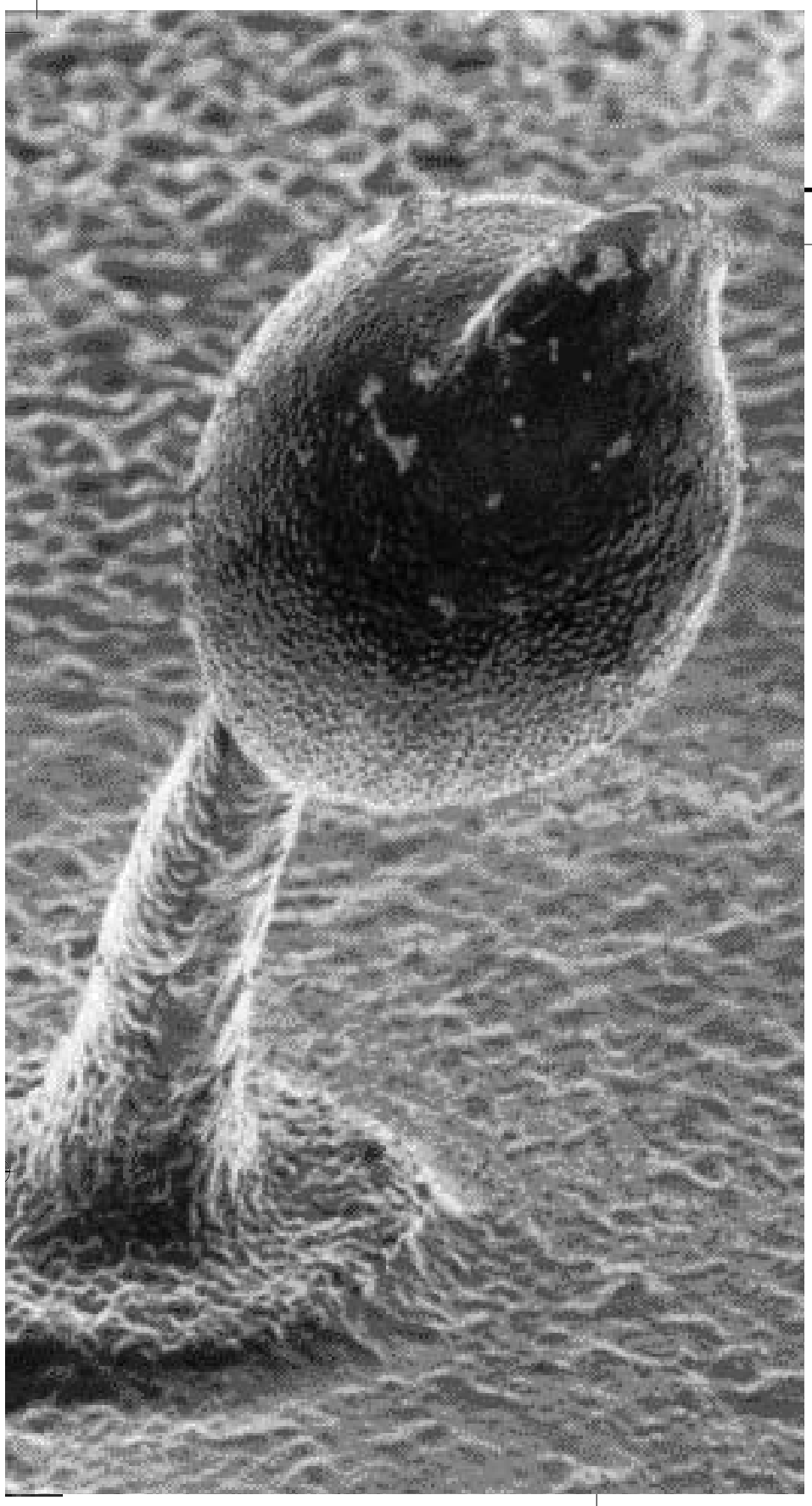


"A leader in his field": Paul Fisher (above left) has been ignored in Australia but his research into Dictyostelium (a spore of the species is shown above) has won him acclaim abroad.

"Fisher is a truly international scientist, a leader in his field whose work is recognised throughout the world," she says.

In part because of this work, slime mould labs are hatching internationally. But in Australia they're closing shop. Our medical funding bodies won't fund slime mould research because of a crucial question: is slime mould plant or animal? Or rather, they won't fund research because of the *answer* to this question.

As political as it is scientific (there's territorialism in science funding), the plant-animal question has provoked rancorous debate for decades. The slime mould cell makes its own cellulose, so it must be a plant. Yet sometimes it looks and moves like an animal and, along with its fellow cells, it can form something like a fungus.

A single-celled creature, it also forms multicellular bodies that, at different stages in its life cycle, resemble many things. A changeling, a shape-shifter, the slime mould defies traditional classification.

This is what confuses science funding bodies, which have strict ideas on what class of creature is suitable for medical research. So unclassifiable was the slime mould, it spawned a rethinking of evolutionary theories, a pile of doctoral conferences and theses, and a new poster child for creationists, whose websites claim that slime moulds once and for all settle the creationist debate.

God "put slime moulds here so that we could be in no doubt about the origin of life", say creation evangelists Rodney McQueen and David Catchpoole on the Answers in Genesis website. "As Romans 1:20 implies, He would create some things which would thwart man's attempts to explain everything by evolution." This, says Fisher, a practising Christian, "is utter rubbish … everyone now agrees they're neither plant nor animal but in a separate lineage".

But creationists have this much right: the slime mould baffles evolutionary biologists. It has no fossil record. The creature is maddeningly elusive, unspeakably inhuman, but more like us than we'd care to think. "So much like us," says Fisher, "that if you stick one under a microscope with human white blood cells, you can't spot the difference."

Within the dour walls of La Trobe's Bundoora campus, Fisher's slime mould lab is full of light and activity, vessels of coloured fluids, trembling machines and a chattering radio. It's here, in this unassuming space, that some momentous discoveries have been made: among them, potential treatments for our most serious genetic diseases.

This specimen's passage across a Petri dish, Fisher explains, shows us how. In the Petri dish is a fissured gelatinous moonscape, a community of slime mould cells growing on a lawn of bacteria.

Fisher moves purposefully around the lab with the dish and slides it under a microscope. "If you're patient and watch carefully," he says, "you can see the shape changing and things moving around." This movement is caused by cellular signalling. Molecular signals such as steroids, hormones and enzymes issue instructions to cells, telling them when to doze off, when to move about, how to grow or transform. There's a mighty cast of these characters in our bodies, playing a host of different roles at different times.

That's the problem with experimental biology. It's often stymied by the number of variables in a creature. Give a lab mouse cancerous genes, and we might observe how disease symptoms play out, but a million other molecular agents have intervened in the plot. Stress hormones from the creature's adrenal glands, perhaps. Peptides from its brain. So the mouse can't give us "pure" information about disease.

Very early on in his student days, Fisher recognised this. The trick, he figured, would be to find a model organism – a microbial "guinea pig" – with none of these variables. A very simple, single-celled creature could tell us how genes affect cell behaviour. A yeast cell wouldn't do – it doesn't move or communicate; it simply reproduces to form a mound in the Petri dish. And a bacterium has little in common with human cells. Fisher would have to find a single-celled creature that behaved as human cells do. One that shares a good deal of our DNA.

As an undergraduate in the early 1970s, Fisher set about looking for such a model organism. Determined to be the first in his family to gain a tertiary education, he supplemented his $5-a-week scholarship by working as a mail sorter, a storeman and packer, and as a sales clerk for a wallpaper business. "I'd started uni the year [1972] that Gough Whitlam was elected, when life was full of hope. Employment prospects were bright, universities were growing."

But within a few years, Fisher was recovering in hospital. A vehicle collision with a drunk driver had almost killed him. En route to hospital by ambulance ("It was quite exciting – I had a police escort!"), Fisher observed how paramedics documented the brutal injuries to his organs and bones (today he can still list more than a dozen, precisely) as he lost litres of blood.

Almost a quarter of a year in intensive care and rehabilitation gave him an enduring empathy for those suffering ill-health, and motivated him to find cures. "I wanted to be the one making the fundamental discoveries," Fisher says.

Which led him to the slime mould. Staggering on crutches, he could "[still] carry conical flasks around with medium in them without any real problems". (As he lifts specimens today, he feels residual pins and needles in his fingers; he walks with six steel pins in his hip.) Overcompensating for missed study, he embarked on an almost manic quest for knowledge between work, lectures and pain management. Swotting textbooks on subjects he couldn't cram into his degree, he chanced upon a small chapter on the "Dicty" slime mould.

Fisher had found his model organism.

"They're fantastic little creatures," Fisher says fondly. The *Dictyostelium discoideum* species, or Dicty, is particularly fantastic, because it has a sex life that would make a pornographer blush. For most of us, starvation ranks as one of life's most unerotic experiences, but to the Dicty cells starvation inspires an orgy of epic dimensions.

"When they're starving," Fisher explains above the thrum of the lab, "they become attractive to each other and you get maybe 100,000 of them, all coming together.

"Then," he adds, in words that seem flat on the page but musical to hear, "they form this multicellular organism like a slug, that crawls around!"

Fisher regularly starves his Dicty slime mould cells, seducing them into the slug stage. In this stage, the cells work collaboratively, like a human organ or a body. Yet unlike humans, this body has no brain or central nervous system issuing instructions.

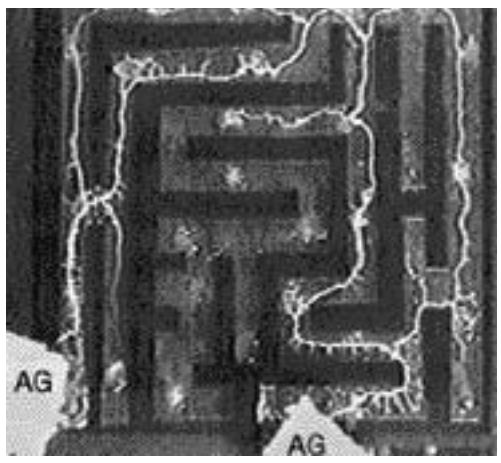
Here's the mystery. In the aggregation of

brainless slime mould cells, no one is marshalling the troops. No one is calling the shots. So what, exactly, is telling this community of cells to behave as one creature? How does it "know" to move and co-operate with its colleagues?

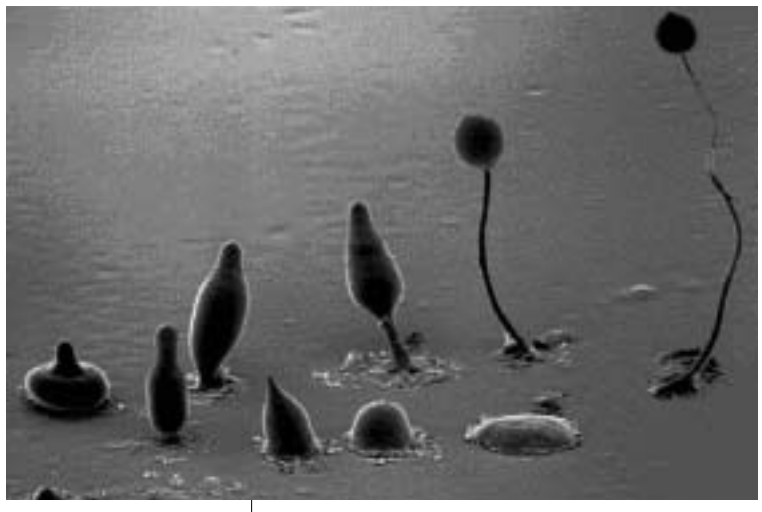
If Fisher could find the genetic answer to these questions, perhaps he could understand what motivates cells to stop communicating with each other (as in degenerative brain or muscle disease) or reproduce too rapidly (as in cancer) or move and heal the body (as in white blood cells to a wound site). If he could isolate the genes needed for Dicty cell movement and communication, and identify the proteins that these genes encode, maybe he could unlock the key to some of our genetic diseases.

That's exactly what he did. Years of conjecture, frustrations, trial and error, clues and dead ends didn't deter him from the final, momentous discovery. But nor did it prepare him for it. "Our first reaction was shock. The next was, well, what does this mean?"

One day, during an experiment to isolate genes needed for slime mould movement, Fisher made a significant discovery: he was the first to find a method to mutate the genes of the slime mould's mitochondrion. These are the very genes that make a slime mould a little bit human.



Intelligent cells? (right) the route taken by a slime mould that wound its way through a complex maze to food. The result of the Japanese experiment astonished researchers; (below) the life stages of the *Dictyostelium discoideum* species.



Years of conjecture, frustrations, trial and error and dead ends didn't deter Fisher from the final, momentous discovery. Nor did it prepare him.

The mitochondrion is an ancient bacterium – a tiny, vestigial creature dwelling within every human cell. And within every slime mould cell. Fisher believes this miniature organ first invaded our ancestors "sometime between the primordial soup and the first multicellular creature". A few billion years ago. Long before the major plant-animal lineages.

Today the mitochondrion plays a mighty role in the human body. The engine-room of each of our cells, it kindles cell respiration and metabolism, it sends out signals, it creates and folds proteins, it helps instruct the cell when to divide and when to die.

But when mutated, it has some sinister traits. Mutant mitochondrial genes can write recipes for an entire chapter of misfortunes, including some forms of dementia, epilepsy and Alzheimer's disease, movement and muscle disorders and strokes. In short, mutant mitochondrial genes are responsible for some of the most debilitating and incurable human diseases.

Many of us inherit some mutant genes but can get through much of our lives without noticing. But mutant genes can reiterate – or "re-express" themselves – from the effects of lifestyle, ageing, diet, stress, environment and each person's unique biochemistry. So it's a tricky business trying to tease out the precise genetic cause and the symptomatic effect of a degenerative disease.

Here's where the Dicty slime mould helps out. "Dicty doesn't age," says Fisher. "It's immortal. In the right conditions you could keep one alive indefinitely."

So when Dicty's mitochondrial genes are mutated to replicate human diseases, we can "chart the genetic cause and the cellular outcome" with absolute purity. Human factors such as ageing, smoking, road-rage and in-laws have no place in the life of a slime mould.

When Fisher mutated Dicty's mitochondrial genes, he had in effect (if not in actuality) given the slime mould some forms of Alzheimer's, Parkinson's, blindness, deafness, dementia, migraine and epilepsy. He'd given heart disease to a heartless creature. Diabetes to a creature without a pancreas. And so on.

All these diseases come from the same set of genetic mutations. "The different clinical diseases are different outward expressions of the same underlying genetic cause – it all depends on what issues and organs happen to be most affected in any individual," he says.

His lab is now the only one in the world where almost any mitochondrial disease can be created at will. Respected Japanese neuroscientist Professor Yasuo Maeda describes Fisher's system as "outstanding … of great importance not only for basic biology, but in investigations of mechanisms leading to various mitochondrial diseases … critical to developing new treatments".

Finding the genetic cause is one thing. But the next step is to identify the molecular enemy. In other words, what do those mutant genes encode? Willing to travel wherever their evidence guided them, Fisher and his colleagues gradually learned that mutated mitochondrial genes activate a protein that scrambles cell communication.

For the sake of science publishing protocol (Fisher is yet to publish this research), let's call it Protein X. "Protein X," Fisher explains, "triggers chemical messages that issue instructions to cells. All cells communicate by sensing chemical messages from other cells, such as hormones [themselves proteins], or extracellular signals such as light. They detect and process these signals and respond accordingly. This process involves cascades of biochemical events. Each event is triggered by the one preceding it."

This cascade of biochemical events is what's happening in our brain cells when we think, and in our organs when they digest, produce insulin, pump blood or take in oxygen. It's what's happening in our white blood cells when they move to a wound site, or our muscles when they follow instructions issued from the brain.

But when a cell is born with mitochondrial disease, this cascade of events becomes scrambled. In Fisher's mutant slime mould cells (those with mitochondrial disease), Protein X became chronically overactivated. So the cells became bludgeoned with instructions.

"It's like having someone chattering loudly in your ear when you're trying to listen to an important phone message," says Fisher.

"The cell loses track of other messages it should be responding to. If a nerve cell can't listen to a signal from another nerve cell it can't respond any more. This is particular to mitochondrial diseases."

Given a mitochondrial disease, the mutant Dicty cells "forget" how to move in their routine straight passage across the Petri dish, instead becoming disoriented – the very same way brain cells lose track of memory, or muscle cells misremember how to move, or maybe how pancreas cells forget how to make insulin.

Healthy, unmutated slime mould cells, given doses of Protein X, also demonstrate this confusion. Protein X would seem to be the culprit.

But what of treatments? Would it be possible to ease this cellular mayhem?

Fisher's conjecture was vindicated in a further experiment. Given an *inhibitor* of Protein X, the mutant slime mould's communication lines cleared. Its passage across the Petri dish returned to its normal straight path – its symptoms were gone.

"We can create a molecular inhibitor of the Protein X enzyme that cures symptoms of mitochondrial disease," says Fisher. All the available evidence from labs around the world suggest the same is true for humans.

His findings, his new methods and his lab's plotting of the world's first "genetic dose response curve" (a molecular street directory) are breakthroughs that have made Fisher a "respected and trusted leader worldwide", says Birmingham University cell biologist, Professor Robert Insall. The US National Cancer Institute's principal investigator, Dr Carole Parent, says Fisher's work marks him as "an outstanding scientist who has made significant breakthroughs" for the treatment of disease.

Despite global recognition and praise for his work, Fisher's lab can't get funding to complete its research. American colleagues have told Fisher that if he moved his lab to the United States, where slime mould is a respected model organism, he would have millions of dollars of funding a year.

TOSKIYUKI NAKAGAKI; LARRY BLANTON

"The more original and innovative your work," says Fisher, "the less likely it is to be funded. Australia is a fantastic place to live, but it's a terrible place to be a scientist."

Paul Fisher has never owned a car. Only now, in his current professorship as chair of La Trobe's department of microbiology, can he afford one (he still cycles to work). His lab houses three microscopes – one he bought in 1986 from a grant, one donated from a colleague, and one he "dug out of the basement". It's broken. He has to fund his own attendance at conferences. Very often he can't afford to.

The reasons for this are at the very core of what it means to be a scientist in Australia. The pursuit of science for knowledge (a motivation beyond profit or recognition) doesn't pay.

The mounting body of knowledge in Fisher's lab is shared universally; it's not subject to patents or intellectual property rights or commercial-in-confidence clauses. Nor is it massaged by fiscal interests. "Paul's most remarkable quality," says the US National Cancer Institute's Carole Parent, "is his global approach to scientific discovery. Paul is generous and readily makes [his work] available to the community. In the midst of his busy schedule, he periodically visits outside laboratories to keep them abreast of novel techniques and thinking."

To Fisher, sharing his knowledge is a matter of moral imperative. "It's unethical to deny knowledge," he says. While genetically engineering and patenting organisms for profit is ethically debatable, the only ethical implications of conducting "human" experiments on slime moulds, he believes, "are positive. If one can answer questions with Dicty then one can avoid a lot of animal work."

But here's the perverse politics of the plant-animal debate that raged around slime mould. Australia's National Health and Medical Research Council (NHMRC) "simply doesn't fund fundamental biomedical research on non-animal models", says Fisher. Because slime mould didn't wind up in the "animal" classification, it's out of the funding race.

"It is brutal getting simple model research funded by the NHMRC," says microbiologist Dr Keith Williams, who holds an Order of Australia for services to science. "[Because] the world tends to forget where original discoveries were made. Core discoveries about cancer genes came from studies on yeast. Core discoveries about how cells adhere were made from Dicty slime mould. When these studies were replicated in mice, the world thought this is where the discovery was made."

Why, then, wasn't Dicty funded by the NHMRC? Its National Research Priorities Implementation Plan, released in 2003, acknowledges that funding basic research like Fisher's is "difficult … because of limited capacity to evaluate long-term outcomes of research". Research funding in Australia, it says, is "overly complex" and "inadequate" and "the highest quality research is not being funded at an appropriate level".

To resolve problems like this, "the NHMRC is working towards better alignment of research grants with national health and research priorities, without abandoning the innovations that typically come from [basic] scientific research", the chair of the council's research committee, Professor Judith Black, said last month.

The council recently established advisory groups that will consider basic biomedical science, a move Fisher hopes will be promising for slime mould.

While he has never received feedback on the reasons Dicty failed to get funding (and the NHMRC could not explain why to Good Weekend), the council's revised approach promises to deliver better feedback. Other science funding in Australia, such as scholarships, industry grants, and the Federal Government's Australian Research Council, "favours a commercial quick-fix, not a contribution to scientific understanding", Fisher argues.

The last winner of the Nobel prize for medicine, he points out, took six years before the experiment worked; and previous winners, the inventors of in vitro fertilisation, took 20 years. "These people would have no place in our system."

Sufferers of mitochondrial disease may be hankering for treatments, but Australia appears so far to lack the will or the patience to pursue potential treatments involving a model organism with no profile, no profit projection and no listing on the sharemarket. Of the four Australian Dicty labs that sprang up in more optimistic times, only Fisher's survives, largely through the underpaid labour of his graduate students and Fisher's own persistence and resourcefulness.

Overseas, though, it's a different story. There are now 160 Dicty slime mould labs globally, many influenced and informed by Fisher's work. Some have taken on research that Fisher designed but which failed to get funding in Australia. These labs – uncovering the secrets of diseases ranging from cancer to bipolar disorder – are well-funded by medical bodies and private philanthropy on a scale unknown in Australia.

Fisher has rejected overtures from international colleagues to follow the money and move his lab overseas. Here, under pressure to change the focus of his work for the sake of funding, he remains devoted to the slime mould "because I believe in the importance of our work. Because there are no other model organisms as good for answering the questions we're asking."

And because this mysterious, unlikely, primal creature is more like us than some care to consider. By evolutionary chance or intelligent design, our cells, organs and muscles, our ancestors and our descendants are, in many ways, slime moulds writ large. ■