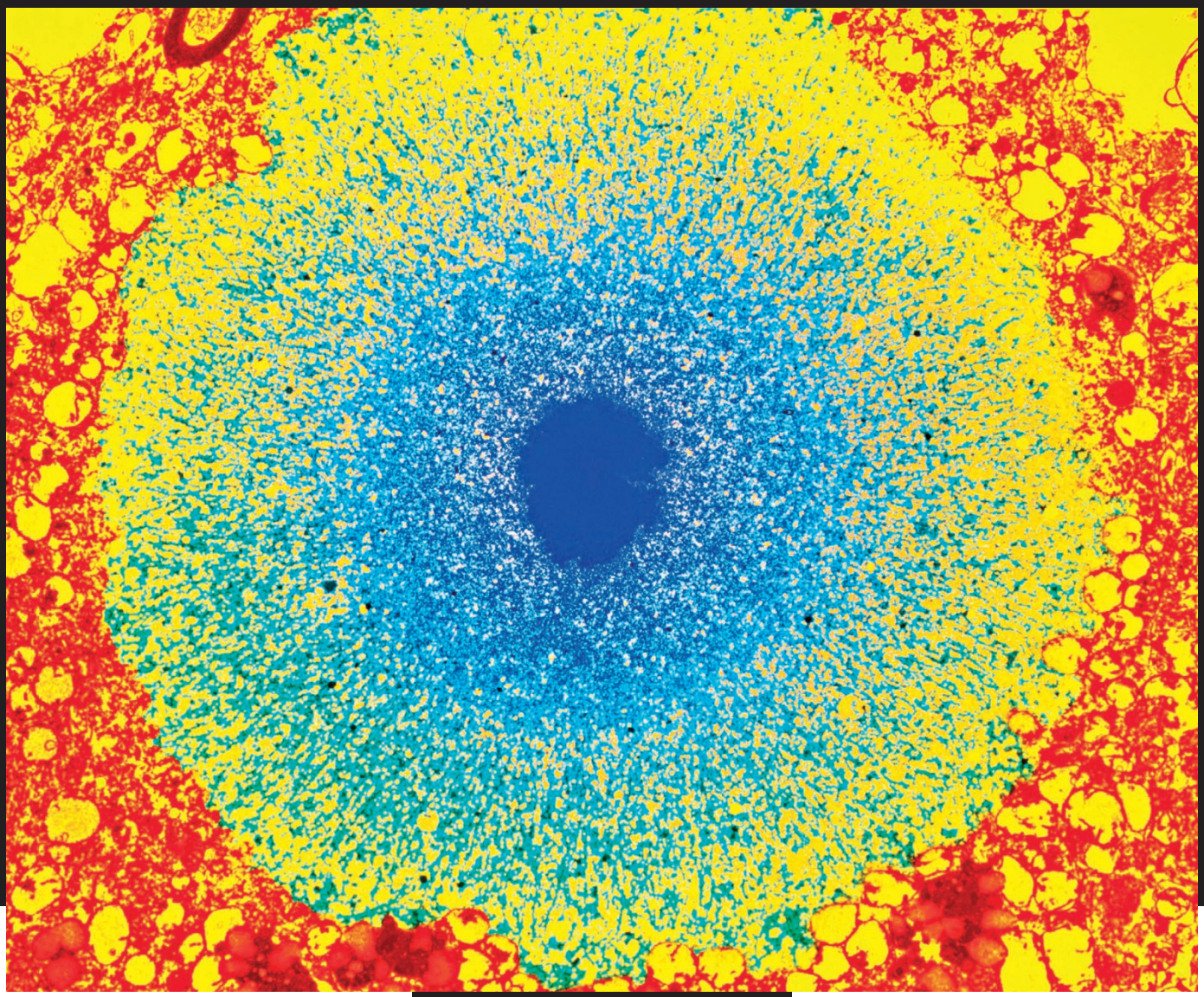
The prion principle



Transmission electron micrograph of a Lewy body, which is made of misfolded α-synuclein protein.

LYSIA FORNO/SPL

BY SIMON MAKIN

A controversial theory that could revolutionize our understanding of Parkinson's disease is gaining ground. But not everybody is convinced that misfolded proteins that spread in the brain are the cause of the disease.

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It was a meeting that they would never forget. Patrik Brundin and Anders Björklund, two Swedish neuroscientists, were attending a symposium at Stockholm's Karolinska Institute. But they were unable to concentrate on the talks — they had just received some intriguing slides of brain tissue taken from a person who'd had Parkinson's disease. During the first coffee break, they pulled fellow neuroscientist Jeff Kordower aside to show him the slides. What Kordower saw that day in August 2007 has had a profound impact on the way that many researchers now view the fundamental nature of Parkinson's disease.

The images were from someone who had received two grafts of fetal brain tissue. But although the graft tissue was by then no more than 16 years old, the cells did not look 'teenage' — some contained dark spots. "We showed him slides of one of our graft patients and Jeff agreed, these were Lewy bodies," says Brundin, who is at the Van Andel Research Institute in Grand Rapids, Michigan. "He was stunned." Lewy bodies are protein aggregates that are hallmarks of Parkinson's disease. The surprise was that these clumps were in cells that should not have been old enough to develop such pathology.

Kordower, now at Rush University in Chicago, Illinois, had previously examined the brain of someone else who had died four years after similar surgery, yet had not found Lewy bodies in the graft. But he had another brain waiting in his lab from a patient who had lived for 14 years after surgery. When Kordower got back and examined this brain, he too found Lewy bodies in some grafted cells.

When the researchers' findings were published[1,2] in consecutive pages of *Nature Medicine* in 2008, they shocked the field. The papers suggested that, given enough time, Lewy pathology could spread from the host to the graft.

Neurodegenerative diseases such as Parkinson's and Alzheimer's progress slowly. This used to be thought of as a facet of the decline in function that is associated with ageing. But evidence is mounting that, in these disorders, something is spreading in the brain. This revolutionary — and still controversial — idea unifies many neurodegenerative diseases under a shared mechanism. "It's the single most controversial question in the field right now," says neuroscientist David Sulzer of Columbia University in New York City. If the spread theory holds up, not only will it have huge implications for diagnosis and treatment, says Sulzer, "it will transform the understanding of the kind of disorder that Parkinson's is" — and potentially that of all neurodegenerative diseases.

> "It's the single most controversial question in the field right now."

THE PROTEIN PATHOGEN

Lewy bodies were first found in 1912 in the brains of people who'd had Parkinson's (see page S2), but their exact relationship to the disease was unclear until two studies in 1997. First, geneticists identified the gene *SNCA*, which produces a protein called α-synuclein, as a cause of inherited Parkinson's[3]. Then a team at the University of Cambridge, UK, showed that α-synuclein was the main constituent of Lewy bodies[4]. These findings suggested a causal link between Lewy bodies and the disease, and they also put a new villain on the stage: an obscure protein without a known function.

This protein is now thought to have a role in communication between neurons. But it is not the loss of this function that seems to underlie Parkinson's disease. "The common understanding is that its toxic role is due to new properties that occur when α-synuclein is misfolded," says Sulzer. But saying that misfolded α-synuclein is involved is not the same as showing that it spreads. That came with the 2008 graft studies. "It looked like the α-synuclein aggregates spread from the patients' tissues into the grafts," says Michel Goedert, who, along with Maria Grazia Spillantini, led the Cambridge team. "That's quite strong evidence."

There was precedent for the idea that disease can be caused by the spread of misfolded proteins. Back in 1982, biochemist Stanley Prusiner, now director of the Institute for Neurodegenerative Diseases at the University of California, San Francisco, showed that a class of diseases called transmissible spongiform encephalopathies was caused not by a microbe, but by prion protein (PrP)[5]. PrP exists in a healthy form, but causes disease when it misfolds into shapes that induce other PrP molecules to do the same — and so becomes self-propagating. As clusters of misfolded PrP spread, they damage and ultimately kill cells. Although controversial at the time, the discovery eventually earned Prusiner a Nobel prize in 1997.

Prusiner has long argued that most neurodegenerative diseases are prion diseases, but others have pointed to differences between them — notably infectivity. Prion diseases are considered to be transmissible, partly because of infectious animal forms such as scrapie, but also because of one highly publicized animal-to-human infection event, in which more than 200 people, mainly in the United Kingdom, developed variant Creutzfeldt–Jakob disease (vCJD) from eating infected beef in the 1990s. By contrast, there is no evidence of person-to-person transmission of diseases such as Parkinson's. As a result, some researchers are wary of classifying α-synuclein as a prion. "You can call it prion-like, but not a prion," says neuroscientist Virginia Lee of the University of Pennsylvania in Philadelphia.

But there are similarities between the conditions. Most cases of CJD, the most common prion disease found in humans, are not caused by infections: around 85% are idiopathic (arising spontaneously), which is similar to the incidence of idiopathic Parkinson's. Fewer than 1% of CJD cases result from infection, and they all occurred under unusual circumstances, including a handful involving neurosurgical tools. "The reason people are hesitant to use the term 'prion' is that it carries such baggage — mainly because it invokes memories of 'mad cow' disease in the UK," says biochemist Joel Watts, who worked in Prusiner's lab before starting his own at the University of Toronto in Canada.

Proponents of the spread theory also point to work in 2003 by a team led by anatomists Heiko Braak and Kelly Del Tredici at Goethe University in Frankfurt, Germany. The team examined post-mortem tissue from people who had either been diagnosed with Parkinson's, had no symptoms but had Lewy bodies, or were healthy controls[6]. Based on the distribution of Lewy bodies, the group proposed that Parkinson's progresses through the nervous system in stages, starting with the brain's olfactory bulb (which receives input from the nose) and the part of the brainstem that connects the brain to the nerves that line the gut. Parkinson's then creeps up the brainstem to the midbrain (including a region called the substantia nigra, where it causes motor symptoms), then to the lower forebrain, and eventually the cortex (see

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'Pathological processes'). The spread from nose and gut to regions of the brainstem that control autonomic functions seems to explain the symptoms that often precede motor impairment, including loss of smell (anosmia), constipation and problems swallowing (see page S5).

The suggestion was that a virus or "a prion-like pathogen", consisting of misfolded α-synuclein, could be responsible for the staged progression, says Del Tredici. But the idea received scant attention until the *Nature Medicine* studies five years later.

PURSUING PROOF

Armed with a suspect for Parkinson's, and a theory to test regarding α-synuclein's prion-like role in disease progression, researchers began to investigate in earnest whether the protein really could spread.

Test-tube experiments had shown that single molecules of α-synuclein can misfold into shapes that induce other monomers to do likewise, and that these cluster into larger oligomers — ultimately forming filament-like structures called fibrils. Some of these aggregates break apart to form seeds, recruiting more monomers and accelerating the process.

A major obstacle that the spread theory had to overcome was the fact that α-synuclein was thought to exist only inside cells. This was in contrast with PrP and amyloid-β (the protein suspect in Alzheimer's disease), which both exist outside cells. Cell-culture studies have since suggested that neurons can absorb and release α-synuclein, although how this happens is still unclear.

So α-synuclein can misfold and aggregate, and can probably get in and out of neurons. But could it actually spread in the brain? Lee's lab tackled this question in 2012. "It's a very simple model," Lee says. "You just take a regular mouse and inject a bit of this misfolded protein that we make in test tubes." Lee's team targeted the striatum, the brain region where the fetal grafts were implanted[7]. The striatum works with the substantia nigra to control movement, and this circuitry relies on the neurotransmitter dopamine. Over six months, the researchers saw a build-up of Lewy-like pathology in brain regions connected to the striatum, substantial death of neurons in the substantia nigra, reduced dopamine in the striatum and, ultimately, motor impairment — a reproduction of the major features of Parkinson's disease.

The next step was to move from mice to non-human primates. In 2014, a team at the University of Barcelona in Spain injected Lewy bodies extracted from human brains into the substantia nigra and striatum of four macaque monkeys[8]. Over 14 months the researchers observed gradual neuronal degeneration, first of the long axons that extend from neurons in the substantia nigra to the striatum, then of the substantia nigra neurons themselves. It was a small sample, and they did not report any functional changes, but nevertheless this was the first indication that α-synuclein from the brains of people who'd had Parkinson's can trigger Parkinson's-like pathology in a close evolutionary relative.

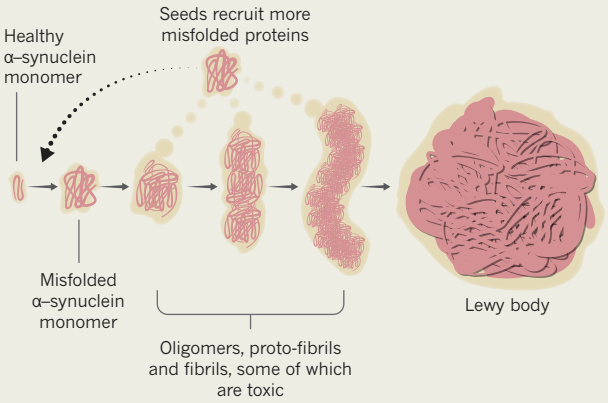
But Parkinson's does not start in the striatum. So a team led by Brundin has instead targeted the olfactory bulb — one of the disease's proposed origins, according to the Braak staging hypothesis. The team's most recent study, a collaboration with Lee's group, used synthetic fibrils injected into mice[9]. The researchers saw a gradually expanding distribution of Lewy-like deposits, which Brundin says is evidence that α-synuclein spreads through neural connections. "We see spreading over 40 brain regions, over 1 year," he says. "It exactly follows the anatomical pathways in the olfactory system." The team also measured impairments in the animals' sense of smell, which Brundin says "supports the idea that the disease starts here, you develop anosmia, then ten years later you get Parkinson's".

Not everybody is convinced that there is a direct link between Lewy bodies and neurodegeneration, however. Neurodegeneration in Parkinson's disease can occur before Lewy bodies appear, and Lewy bodies

PATHOLOGICAL PROCESSES

The α-synuclein protein aggregates to form Lewy bodies, which then seem to spread through the brain as Parkinson's disease progresses.

As individual α-synuclein proteins misfold, they form clumps of increasing size, some of which break apart and seed new aggregates. The end stage is the Lewy body, the hallmark of Parkinson's disease. Some researchers think the aggregation into Lewy bodies limits the damage caused by α-synuclein.



According to the Braak staging hypothesis, as Parkinson's disease progresses, Lewy bodies are found in more areas of the brain in a characteristic pattern of spread.

Stages 1 & 2
When Lewy bodies are confined to the brainstem and olfactory bulb, the disease is in its preclinical stage, mainly affecting the autonomic systems and the sense of smell.

Stages 3 & 4
Lewy bodies are found in the mesotemporal cortex and midbrain, and clinical signs include sleep disturbances and motor symptoms.

Stages 5 & 6
In the later stages, Lewy bodies can be found in the cerebral cortex, and symptoms now include emotional and cognitive impairment.

Stage 1 · Stage 2 · Stage 3 · Stage 4 · Stage 5 · Stage 6

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sometimes show up in cells that do not die. This has led researchers to propose that it is the smaller α-synuclein aggregates that are the most toxic, and that a Lewy body represents a form of damage limitation.

A JIGSAW PUZZLE

One of the most important arguments centres on the distribution of Lewy bodies. If α-synuclein simply follows neural pathways, it would be expected to spread to all connected brain regions. Instead, only certain types of neuron seem to be vulnerable. This observation leads some researchers to favour an older idea: that some cells succumb earlier to attack by misfolded α-synuclein because they are inherently weaker. This vulnerability could be due to certain neurons having less-efficient waste-clearance systems, or because of their overworked energy-producing mitochondria. The immune system also seems to be involved: α-synuclein can trigger inflammation, and Sulzer's group has shown that, when this happens, neurons can mark themselves for execution by immune cells[10]. This process releases the cell's α-synuclein, so it could create a vicious cycle of inflammation.

Some researchers go even further and suggest that α-synuclein spread is a red herring. "If the spread is toxic in some cells but not others, then the key factor determining whether a neuron dies has to be something other than misfolded α-synuclein," argues physiologist Jim Surmeier of Northwestern University in Chicago, Illinois. And if that key factor is the vulnerability of a neuron, he says, then it's not clear what the spread theory adds to an understanding of the cause of Parkinson's.

Surmeier has his own ideas about neuronal vulnerability in Parkinson's. All commonly affected types of neuron — at least those that have been studied in detail — share two features. They have long, highly branched axons that have lots of synapses, and so probably contain high levels of α-synuclein. They are also what he calls chatterboxes — power hungry and constantly active. "What links the cells that degenerate in Parkinson's is that they lead stressful lives," says Surmeier. "It wouldn't be surprising if these cells were prone to α-synuclein aggregation; the issue is whether that's induced externally or internally."

If the energy and waste-disposal systems of certain cells are stressed, then that might be enough to explain the anatomical pattern of disease — with inflammation delivering the fatal blow. Most of the Parkinson's genes so far discovered have related to mitochondria and waste-clearance systems, which supports this model. These processes all decline over time, which would also account for age being the biggest risk factor for Parkinson's disease.

Regardless of whether α-synuclein aggregates spread, the protein is clearly involved in the progression of Parkinson's disease. The fact that processes such as waste disposal, energy production and inflammation are also implicated does not dislodge the main villain, because most of these processes also interact with α-synuclein. Sulzer was part of a team that showed that α-synuclein reacts with dopamine to create a new form of α-synuclein that blocks an important waste-clearance system[11]. This could explain why dopamine neurons are especially vulnerable in Parkinson's disease. "These might look like different issues, but they could all be related," says Sulzer. "We have clues about how some of them fit together, but it's a jigsaw puzzle right now."

Important pieces of that puzzle, relating to the spread theory, are not well defined. For instance, if aggregates do move around the brain, it is not yet clear how they are released and taken up by cells. "Ninety-eight per cent of α-synuclein in your brain is inside neurons, in a compartment, the cytoplasm, and isn't naturally secreted," says neurologist Dennis Selkoe of Harvard Medical School in Boston, Massachusetts. What's more, Selkoe questions whether aggregates spread at all. "Does the next cell down definitely get material from the upstream cell, or does it transform its own?" he asks. In other words, do aggregates spread and cause dysfunction, or does dysfunction promote aggregation? "My guess is both can happen under different conditions," says Sulzer.

THE PROGNOSIS

If the prion theory proves to be true, it will provide many strategies for both the diagnosis and the treatment of Parkinson's disease. Early detection, in particular, might be possible if misfolded α-synuclein can be detected in the blood or cerebrospinal fluid. "We could design assays based on things we've had for years in the prion field, based on amplification of misfolded proteins," says Watts. Techniques for tracking the spread of α-synuclein are in the works — other neurodegenerative diseases already have imaging molecules that bind to aggregated proteins, including amyloid-β and tau in Alzheimer's. Efforts to develop the same for α-synuclein are under way.

> "We have clues about how some of them fit together, but it's a jigsaw puzzle."

The ultimate aim is to develop a disease-modifying treatment. "The implication of this knowledge is that we may have a new therapeutic target, which is extracellular synuclein," says Brundin. If α-synuclein does move between cells, it may be more accessible to drugs such as antibodies that are tricky to get into cells. A 2014 study from Lee's group suggested that such an approach holds promise[12]. The researchers seeded the brains of mice with synthetic fibrils, then immediately injected them with α-synuclein antibodies. This reduced Lewy bodies and neuron loss, and improved motor impairments. "It's an exciting first step," says Lee. Establishing whether this works in humans is a long way off, and much depends on clarifying the importance, and mechanisms, of spread.

Many researchers find the parallels between prion diseases and other neurodegenerative diseases compelling. "It's hard to ignore some of the similarities," says Watts. But regardless of whether α-synuclein does spread, gaining a fuller appreciation of this protein's role in Parkinson's will still advance understanding. "The aggregation of α-synuclein," says Goedert, "is the Rosetta stone of Parkinson's disease." ■

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