Treatment frontiers

Cell replacement, gene therapy, and electrical and optical stimulation for the brain — **Kerri Smith** looks to the future of Parkinson's disease therapies.

In February 1985, a team of Scandinavian scientists reported the results of a radical new treatment for Parkinson's disease. Erik-Olof Backlund and his group transplanted cells from the adrenal gland, a source of the neurochemical dopamine, into the brains of patients[1]. Dopamine is the chemical that patients with Parkinson's disease are missing, because the brain cells that produce it gradually die.

Backlund's trial aimed to fill the hole left by the dying neurons. It marked the first time that dopaminergic tissue had been transplanted in the human brain. As the authors cautiously wrote in the *Journal of Neurosurgery*, "some rewarding effects were registered".

That was 25 years ago. Today, cell-replacement therapy for Parkinson's disease is notable for its absence. In fact, no currently available treatments for Parkinson's disease actually do anything for the underlying disorder. "Nothing changes the natural progression of Parkinson's disease once it's established," observes Roger Barker, a cell-replacement specialist at the University of Cambridge.

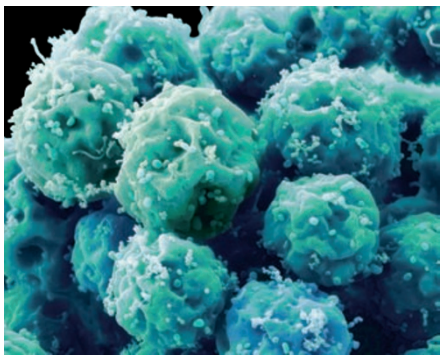
The mainstay of Parkinson's disease therapy is the drug levodopa (L-DOPA), a dopamine replacement that has been in use since the 1960s (see page S6). "L-DOPA helps the symptoms but really does little or nothing for the underlying illness," says Andres Lozano, a neurosurgeon and Parkinson's disease specialist at the University of Toronto. The drug also has side effects, such as nausea, confusion and — with prolonged use — a reappearance of uncontrolled movement, called dyskinesia.

Researchers around the world are working to add new therapies to the time-honoured but imperfect L-DOPA treatment. In the next few years, a number of opportunities are predicted to swim into focus.

The next five years

L-DOPA add-ons. The pipelines of many pharmaceutical companies include supplemental drugs for the classic L-DOPA treatment. L-DOPA is commonly administered with other compounds that improve its pharmacokinetic properties and ameliorate its side effects. The biggest problem, however, is that over time patients become resistant to L-DOPA.

Several drugs in development aim to address this problem. Many of them target the dopaminergic pathways in the brain's basal ganglia — an area in the middle of the brain that controls movement initiation. The basal ganglia, which comprises the striatum, the subthalamic nucleus (STN) and the pallidum, usually receives inputs from a tiny group of cells nearby called the substantia nigra; however, the substantia nigra is exactly where dopamine cells die in Parkinson's disease. As a consequence, the pathways throughout the basal ganglia are disturbed.



Many researchers believe that embryonic stem cells have potential to treat Parkinson's disease.

The members of one group of compounds, adenosine A2a receptor antagonists, interact with the specific dopamine receptor subtype D2 in the basal ganglia, making it more sensitive to dopamine. The rationale is that this will increase the effects of L-DOPA, and thus boost its efficiency and duration.

Adenosine A2a receptor antagonists have been shown to reduce Parkinson's disease-like features in animal studies, and are showing promise in human trials. At the annual meeting of the American Academy of Neurology (AAN), which took place earlier this year in Toronto, a team led by Kevin Black at the Washington University in St Louis School of Medicine announced positive results from a phase IIa trial of one such drug, called SYN-115, from the silos of Synosia Therapeutics. The team showed that the drug crossed the blood–brain barrier and calmed activity in affected brain regions. Black confirmed the clinical benefits: treated patients' scores on the standard Unified Parkinson's Disease Rating Scale (UPDRS) — used to assess motor activity — were better than those of patients who had not received the drug. They are now planning phase IIb trials.

Dopamine is not everything. Other emerging classes of drugs take aim not at dopamine but at other brain chemicals also affected by dopamine depletion. The loss of dopamine cells in the substantia nigra effectively lifts the brakes on activity in that area. Cells of several different types, including those that affect glutamate — a neurochemical found throughout the brain — start to fire uncontrollably. This excess of excitation worsens motor symptoms. Researchers hope that by reducing glutamate levels in appropriate areas, these side effects might be avoided.

One glutamate receptor being targeted is mGluR5. Drugs that affect this receptor are already in development at several pharmaceutical companies, including Roche and Novartis, as a treatment for Fragile X syndrome — a genetic disorder linked with cognitive impairment and autism. By cooling the activity of this receptor in Parkinson's disease it should be possible to get the network back in check. This action might even allow L-DOPA to be used at a higher dose.

Biotechnology firm Addex Pharmaceuticals is developing mGluR5 modulators, and has already shown that several are safe in patients. One promising candidate, currently known as ADX48621, is soon to enter phase II trials for Parkinson's disease. The firm has other mGluR5 modulators in phase II trials for anxiety and depression, which might have additional benefits for Parkinson's disease patients away from the motor circuits of the brain, said Addex's chief medical officer Charlotte Keywood in a presentation at the AAN meeting.

These other indications are not just a throwaway line from Keywood. Although the dopamine pathway in the basal ganglia plays an important and well-established role, "it's not the whole story," says Barker. Patients often suffer from depression, cognitive problems, and impairments in balance and gait, on top of the classical motor symptoms. "That's the future major problem in Parkinson's disease," Barker says. "Cognitive problems come to dominate the clinical picture."

There are tools that might be adapted to treat these non-motor symptoms. Deep brain stimulation (DBS) is already well established for motor-symptom control. With DBS, patients who are not responding well to drug treatment have an electrode surgically inserted in either the STN or the nearby internal globus pallidus, both of which are components of the

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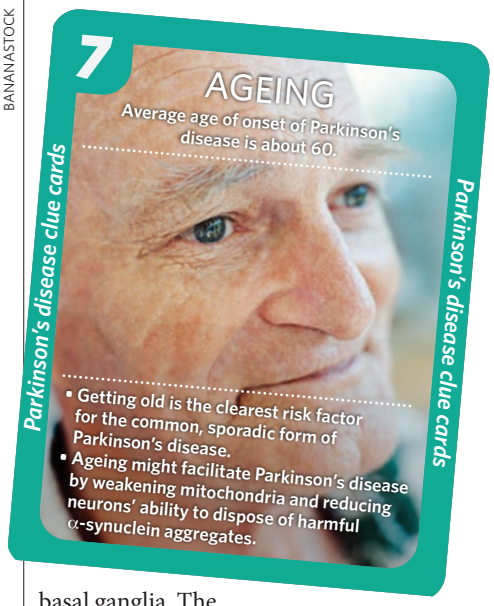


basal ganglia. The electrode is connected to an implanted battery pack, which stimulates the brain regions back into action. More than 50,000 Parkinson's disease patients worldwide have already undergone the DBS procedure.

Lozano and his team are investigating whether DBS can have beneficial effects on non-motor symptoms. To do this, they place electrodes in different areas of the brain that relate to each of these symptoms. For depression, the target is the cingulate gyrus — a crevice near to the middle of the brain where the two hemispheres are sandwiched together. A 2008 study of 20 patients with treatment-resistant depression suggested that, barely six months after surgery, more than 50% had responded to DBS of the cingulate gyrus and around 35% met the criteria for being in remission — benefits that were largely maintained at 12 months (ref. 2).

"Everybody focuses on tremor and stiffness and slowness. But [these devices] also deal with cognitive and limbic functions," says Michael Okun, a neurologist at the University of Florida who specializes in brain stimulation, and who is unconnected with Lozano's team. Because DBS is already in clinical use, approval for new uses in Parkinson's disease should be swift, Okun adds.

The next 5 to 10 years

Stimulating research, stimulating therapy. DBS might be an established treatment for Parkinson's disease, but its mechanism of action is currently unknown. Understanding how it works should be a priority for researchers, says Okun. "We need to understand what's going on at the neuronal level — what does it do to the network, physiologically, chemically?"

Karl Deisseroth, a psychiatrist and bioengineer at Stanford University in California, is starting to do just that. He is one of the pioneers of an experimental technique that allows researchers to turn specific neurons on and off in order to probe the function of different cell types and circuits. Called optogenetics, it involves plugging a light-sensitive protein into brain cells of interest, then switching them on or off with pulses of light (Box 1).

Among other things, Deisseroth has been using this technique to unpick how brain stimulation improves the symptoms of Parkinson's disease. Last year, he and his Stanford team used optogenetics in parkinsonian rats to pinpoint which brain cells, when activated, yielded most therapeutic effect[3]. Deisseroth is optimistic about brain-stimulation treatments because, unlike drugs, they can be precisely targeted to particular brain areas. But, he says, "they need to be better guided by deep insight into the circuitry. And a great deal of that will come from optogenetics."

Looking ahead, it might not always be necessary to insert electrodes deep into the brain. Deisseroth is convinced that within the next decade, stimulation might be successfully administered externally. He has been studying a non-invasive technique called transcranial magnetic stimulation (TMS), with the hope of using it to prod parkinsonian brain circuits into normal action (Box 2). His team has found connections between the STN, which is a popular target for DBS, and the premotor cortex on the brain's surface — meaning that clinicians could stimulate the premotor cortex as a way into the system. "We're already guiding our treatments in humans based on this insight — it's already starting to affect therapy," he says.

Farther away is the idea of using optogenetics itself as a treatment for Parkinson's disease. That would involve integrating light-sensitive proteins into particular neurons in the human brain and stimulating them with light — in a similar manner to DBS. Deisseroth estimates that the first optogenetic trials in patients will begin within 10 years. He adds, "I tell people not to hold their breath. What you can hold your breath for are the insights — what's wrong with the circuit, how is the circuit not performing?"

The next 10 to 15 years

Genetics: new knowledge and new therapies. Geneticists continue to catalogue genes that can be linked with Parkinson's disease[4–6]. Such scans do not, however, reveal what these fingered genes do, the circuit they function in or whether they are good targets for gene therapy. Knitting together a checklist of genes into a circuit has turned out to be a much more difficult task than researchers expected, says Asa Abeliovich, a molecular biologist at Columbia University Medical Center in New York. "It's not gone so well yet," Abeliovich confesses.

Even if researchers arm themselves with knowledge of the genetic circuitry, it will still need to be leveraged into therapy. Work on other neurodegenerative diseases has proved that this is no mean feat. The gene responsible for Huntington's disease was identified in 1993 (ref. 7), but gene therapy still eludes researchers. Huntington's disease is a monogenic condition moreover, whereas Parkinson's disease presents a more complicated genetic picture (see page S2). "The translation of all these interesting findings hasn't really taken place," says Christine Klein, who studies the genetics of Parkinson's disease at the University of Luebeck in Germany[8]. "When you look at current therapy, there's not even a hint of any of the major advances that we've witnessed over the years."

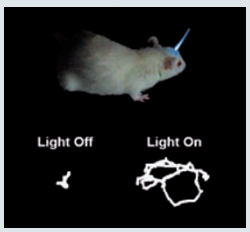
Add to that the fact that many cases of Parkinson's disease develop without any apparent genetic contribution — what clinicians refer to as sporadic or idiopathic Parkinson's disease.

KARL DEISSEROTH, VIVIANA GRADINARU, MURTAZA MOGRI

Box 1 | Light box

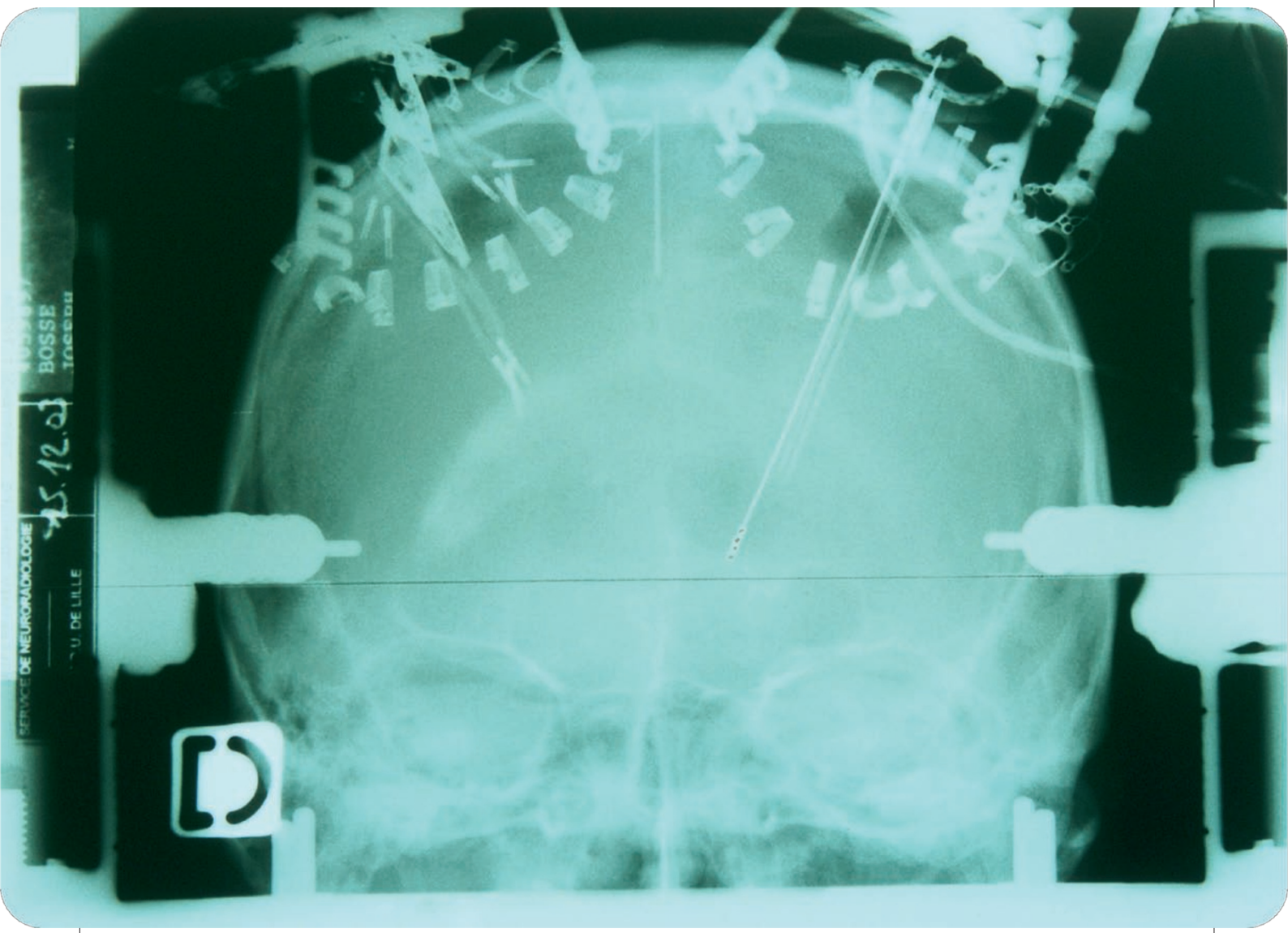
Neuroscientists have algae to thank for the newest addition to their toolkit, as some species harbour light-sensitive proteins called opsins. Certain wavelengths of light cause the opsins to open a channel, others cause them to close. Scientists realized that if they could get opsins into neurons, they could manipulate them with light.

Most *in vivo* experiments so far have been conducted in mice and rats. Genes for these opsins are inserted into specially developed viral vectors, which are in turn injected into target cells in the brain, where they integrate their payload with the host cell DNA. A fibre-optic cable is also inserted to deliver light that, depending on its wavelength, will either open a channel and activate the neurons or close a channel to suppress them.



Optogenetic stimulation increases mobility of a parkinsonian mouse.

A recent study from a team led by Anatol Kreitzer at the University of California, San Francisco used optogenetics to confirm the theory concerning which parts of the brain are affected in Parkinson's disease, and showed that stimulating the circuit in the right way can alleviate parkinsonian symptoms in rodents[16].



Deep brain stimulation for advanced Parkinson's disease involves insertion of electrodes into the brain.

BSIP, ASTIER-CHRU LILLE/SCIENCE PHOTO LIBRARY

Even less is understood about how these cases arise, says Abeliovich.

Despite these caveats, three clinical trials of gene therapy for Parkinson's disease are underway, each using a different gene. In 2008, biotechnology company Neurologix, based in Fort Lee, New Jersey, initiated phase II trials of a gene called *GAD*, which is involved in production of γ-aminobutyric acid (GABA). This is an inhibitory neurotransmitter, and the idea is that — as with glutamate modulation — stimulating it will quieten overactivity in the basal ganglia.

The US National Institutes of Health is currently conducting a phase II gene-therapy trial for glial-derived neurotrophic factor (GDNF), a protein thought to affect how dopamine is made, stored and taken up by cells.

Another biotechnology company, Ceregene, is investigating the role of a related protein called neurturin and its associated gene as a target for gene therapy. In April this year, the company announced further phase II trials of neurturin, even though previous attempts found no evidence of benefit for Parkinson's disease symptoms in patients: two autopsies of patients from the first trial suggested that neurturin had failed to stimulate new dopaminergic connections from the substantia nigra.

The next 15 to 20 years

Cell replacement hits prime time. Instead of introducing genes into the brain, a different approach is to plug new cells into the affected area. In many ways, Parkinson's disease is an ideal candidate for cell replacement: researchers know which cells are dying, what they do and where they live in the brain. It is also a good choice because the disease is relatively common and highly disabling.

Ever since Backlund's early experiments with adrenal gland tissue in the 1980s, several groups have been trying this method to no avail. There are still uncertainties. For one, the underlying cause of cell death is unknown: aberrant proteins, defective mitochondria and ion channels are all under suspicion. "It's not clear which are the key events," Barker admits. These key events might persist, even with new cell implants.

That said, researchers hope that young cells introduced into affected brain areas will remain healthy long enough to be of benefit for Parkinson's disease patients, who are typically 60 years old at disease onset. Researchers are also realistic about the potential of this therapy. "This treatment isn't going to cure people. It might help them a lot but it won't cure them," Barker says.

The earliest cell-replacement trials were performed with fully-formed dopamine cells from embryos. These cells survived well following implant and, in some cases, provided real clinical benefit. Overall the results were mixed, however. Some patients developed worse movement problems after the operation[9,10]. In addition, several autopsies of transplant recipients showed that the fetal tissue had developed signs of pathology — meaning that the disease had transferred from patient to graft[11,12]. There were successes though.

Box 2 | Magnet power

Transcranial magnetic stimulation (TMS) is a way of affecting the activity in the brain without the need for surgery. An electromagnetic coil held outside the skull creates pulses that excite neurons in the target area of the brain, increasing activity and creating a temporary and reversible 'virtual lesion'. TMS is currently used mainly as an experimental technique, but it has been tested as a potential treatment for migraine, depression and other neurological disorders — including short-term relief from the symptoms of Parkinson's disease[17]. For longer term benefit, the kind of weak electrical stimulation given in TMS would need to be administered chronically, via a device sitting on the surface of the skull, for example.

KARL DEISSEROTH, DARCY PADILLA, HHMI

Karl Deisseroth of Stanford University adjusts the position of a TMS coil.

"Transplants, when they work, work fantastically well," says Barker.

With fetal tissue, however, there will always be a supply-side problem. It will never be available in large enough amounts to roll this treatment out on a wide scale, says Olle Lindvall, a neurologist at Lund University Hospital in Sweden. There are practical problems of making and distilling enough for the clinic as well as ethical issues of using material from embryos. "It will never be a clinical treatment even if it worked very well," he predicts.

Embryonic stem cells are one alternative that avoid some, but not all, of these ethical and practical issues. Instead researchers are pinning their hopes on the new kids on the stem cell block: induced pluripotent stem (iPS) cells[13]. These are artificially created stem cells, made by taking a normal body cell and reprogramming it with a cocktail of genes to turn back the clock and give the cell the ability to form any other cell type. In the Parkinson's disease setting, the idea would be to take a skin cell from a patient, reprogramme it and then use another mix of genes to turn it into a dopaminergic neuron.

Because they originate from the patient, these cells would avoid issues of transplant rejection. For this very reason, however, they might also be susceptible to the same disease process. "Would they still have the factors that might lead to them getting sick?" Abeliovich wonders.

Some groups have successfully made and studied neurons derived from iPS cells. A 2008 study by Rudolf Jaenisch and his colleagues at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, showed that neurons made from skin cells could integrate into the correct networks in a rat model of Parkinson's disease and improve symptoms[14]. Abeliovich is still cautious. Animal models of Parkinson's disease, he says, do not exhibit the same symptoms as human patients, so animal trials of stem-cell therapies might not be representative. These trials could therefore present an overly optimistic view of how a therapy might work for humans.

Lindvall thinks that the basic functions of these iPS cells need to be much better understood before they can be transferred to the clinic. He is involved in NeuroStemcell, a European project that aims to create stem cells — both embryonic and iPS — that are safe and functional. "Even if you get the cells you want, it still remains to be shown that those cells function as well as the cells the patient originally had," Lindvall says.

Of course, even the best stem cells would treat only one aspect of Parkinson's disease. "People are hung up on making [dopaminergic] neurons and putting them into the striatum, and at best that's only going to help the dopamine system," says Lozano. Yet by the time the disease is diagnosed, the whole circuit is disrupted. "[Once] there are downstream changes, it's no longer a business-as-usual circuit," he adds. "In my view, it's not going to work."

Present tense

So, what are the biggest hurdles to developing treatments for this condition? "Maybe there are many different diseases which we all call Parkinson's," muses Abeliovich.

This could explain why there is, as yet, no single good animal model — each model developed so far represents only one facet or one genetic mutation in a complex disease process (see page S8)[15]. It could also explain the wide range of responses to treatments like cell replacement, over and above the differences due to patient age, condition, surgical procedure or tissue sample used.

Lozano, by contrast, suggests that many neurodegenerative diseases might have more in common than researchers suspect. "The molecules are different, but the processes that go wrong are sometimes quite similar," he says. "If we make inroads in one, it'll have repercussions in the others."

For many researchers, the future of Parkinson's disease therapy might not be about the number of new treatments, or even the number of different versions of Parkinson's disease, so much as how to design the correct mix for each case. "The horizon in my mind is really the tailoring of the therapy," says Okun. "We shouldn't look at it as a disease, what we should be looking at is each individual patient."

Lozano agrees. "My view is that there's a problem out there and we have to apply everything we know," he says. "We don't know which one of these things, if any, [is] going to work. But the more shots you take on goal the more pucks go in."

Kerri Smith is podcast editor for *Nature*.

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