NEUROSCIENCE

Old Protein Provides New Clue To Nerve Regeneration Puzzle

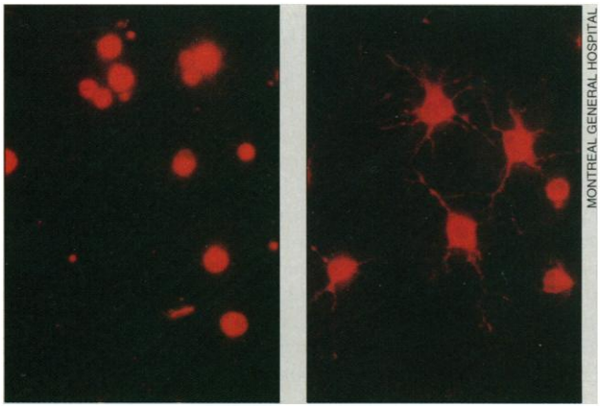
If you cut a nerve in your arm or leg, all is not lost. The cut end of the nerve will send out new shoots called neurites, which grow along the path of the old nerve and restore some neuronal function to the limb. But a cut spinal cord is another matter entirely. While it, too, will sprout tiny neurites, they won't grow across the damaged area. Therein lies a tragic puzzle: Why will peripheral nerves regenerate, while those of the central nervous system (the brain and spinal cord) remain severed, leaving an injured person with permanent neurological damage? Finding the answer to that question promises big rewards, as it may lead to better strategies for treating spinal cord injuries.

Now, two research teams, one including Marie Filbin of the City University of New York and Patrick Doherty and Frank Walsh of Guy's Hospital in London, and the other including Lisa McKerracher, Sam David, Peter Braun, and their colleagues at McGill University in Montreal, have made an advance toward solving this puzzle. The groups independently discovered that a protein known as myelin-associated glycoprotein (MAG), found in the fatty myelin sheath that surrounds nerve fibers, blocks neurite growth in cell culture and may therefore be at least partly responsible for the failure of central neurons to regrow when damaged. The Filbin team reports its results today in the September issue of *Neuron*; the McGill researchers will describe theirs in *Neuron*'s October issue.

The discovery came as a surprise because MAG, which was discovered in the 1970s, had been shown to stimulate rather than inhibit the growth of young neurons—just the opposite of what the new work finds with adult neurons. Although these results in the culture dish have yet to be confirmed in living animals, researchers are hoping that the finding will place them a bit closer to a strategy for mending damaged spinal cords. "The clinical significance is still unclear, but it is definitely a step forward, and very exciting," says developmental neurobiologist Marc Tessier-Lavigne of the Howard Hughes Medical Institute at the University of California, San Francisco.

The search for molecules that block regeneration dates back to the early 1980s, when Albert Aguayo and his colleagues at McGill University found that, although central nervous system (CNS) neurons don't regenerate in nature, they do have the intrinsic ability to do so, if conditions are right. When the researchers transplanted pieces of peripheral nerve across damaged regions of brain and spinal cord in rats, some of the damaged neurons sent neurites across the transplanted "bridge." Only a small percentage of nerve fibers made the trek—but that was better than what the neurons would have achieved on their own.

If CNS neurons are able to regenerate, then something must be stopping them from doing so under normal conditions. At least part of the inhibition seems to come from the myelin that covers CNS nerves: In 1988, Martin Schwab of the University of Zurich found that CNS myelin inhibits neurite growth from a variety of neurons grown in culture. He went on to partially purify an inhibitory protein he calls IN-1, although it couldn't account for all of the effect. When he used antibodies to block IN-1 activity in rats, severed spinal neurons sent out neurites, but only 5% to 10% of the cut neurons crossed the gap. This, says Schwab, may mean that "other [inhibitory] molecules are there."



MONTREAL GENERAL HOSPITAL

No grow. Myelin from the central nervous system keeps the cultured neural cells on the left from putting out neurites.

The Canadian group began searching for those other molecules about 2 years ago. McKerracher and David, who had experience in neuron culture, joined forces with Braun, a biochemist with experience in purifying proteins from myelin. They found two fractions of myelin protein that blocked neurite growth in an immortalized neuronal cell line grown in culture.

In one of these fractions the inhibition seemed to be due to MAG; anti-MAG antibodies, which bind the protein, could completely strip that fraction of all of its inhibitory activity. That was difficult to believe at first, both because of MAG's known ability to stimulate neuronal growth and because it is found in the myelin of both peripheral (PNS) and CNS neurons, which would seem to rule it out as a CNS-specific inhibitor. "People have been suspecting for a while that there will be more than one inhibitory molecule," says Roger Keynes, who studies regeneration and neural development at the University of Cambridge, England. But, he adds, "certainly people weren't thinking about [MAG].... It was definitely a surprise."

Further experiments showed that the surprising result was in fact correct. When the Canadian group tested a pure fragment of MAG protein provided by Robert Dunn, also of McGill, they found that it too blocked neurite growth. Finally, the team used antibodies to remove all the MAG from a preparation of myelin protein and found that neurite growth on the remaining proteins was restored to 63% of what it was in the absence of any myelin protein. They concluded that MAG is a major inhibitor of neurite growth.

Meanwhile, Filbin and her collaborators reached the same conclusion—independently and from a very different direction. Their initial plan was to study the stimulatory effects of MAG that others had observed with young, developing neurons, such as sensory neurons from the dorsal root ganglia (DRG) in the spinal cords of newborn rats. To mimic the way MAG is produced by the cells that make myelin, Filbin first engineered hamster cells to make the protein and then took the cell line to the London labs of Doherty and Walsh, who are experienced in studying neurite growth. The team tested the effects of the MAG-making cells on neurons from the cerebellum of newborn rats, because they happened to have those cells handy at the time. They were shocked to find that cerebellar neurons would not grow over the MAG-producing cells, although they would grow happily on control cells. "We didn't believe it," says Filbin. But the result held up.

The team then repeated the experiment with DRG neurons from newborn rats, as others had done, and found that MAG encouraged growth of those neurons, as expected. But they realized that neuron age can make a difference; Jim Cohen at Guy's Hospital had recently found that adult DRG neurons will not grow on cultured strips of tissue from peripheral nerves, although young DRG neurons will. When Filbin and colleagues tested older DRG neurons from 5- to 7-day-old rats, the neurons refused to grow on the MAG-producing cells.

"The two papers are beautifully complementary," says Tessier-Lavigne. "Together they each answer the major questions raised by the other's work." The Montreal group studied MAG's effects on an immortalized neuronal cell line, whose properties could differ from true neurons. Filbin, Walsh, and Doherty found, however, that the protein has the same effects on neurons that have been recently removed from animals. The Filbin group's experiments could not determine the degree to which MAG contributes to myelin's overall inhibitory effect, an answer that was filled in by the Montreal team, when they showed that MAG accounts for well over half of myelin's inhibitory activity.

But that leaves a third major question: As MAG is present in PNS myelin, why are peripheral nerves able to regenerate? The answer may lie partly in the fact that PNS myelin contains only one tenth as much MAG as does CNS myelin. In addition, MAG is probably not around when peripheral nerves regrow. That's because damaged peripheral nerves undergo a rapid cleanup that removes all of the myelin and debris downstream of the nerve cut. Only after the debris is gone can new neurites grow into the injured area, says Filbin. Cleanup of CNS neurons, on the other hand, is much slower, so MAG-containing myelin stays around much longer after nerve injury.

Still, MAG accounts for only 63% of the neurite inhibition from myelin, although the Montreal group has another, non–MAG-containing protein fraction that also has inhibitory effects. The culprit in that fraction could be Schwab's IN-1, McKerracher says.

If MAG is part of the reason why CNS neurons won't regenerate, then tricks for blocking MAG function may someday be useful for treating spinal cord injuries. But before researchers start thinking along those lines, they first must show that blocking MAG facilitates neuronal regrowth in animals with CNS injuries. The Montreal group has begun such experiments with John Roder of Mount Sinai Hospital in Toronto.

Roder's lab recently made mutant mice that lack a functional MAG gene, and the group plans to see whether CNS nerves can be coaxed to regenerate in the MAG-minus mice. "That's the acid test," says Roder. If MAG passes it, then researchers on the long trek toward nerve regeneration will have taken a significant step forward.

–Marcia Barinaga

SPACE SCIENCE

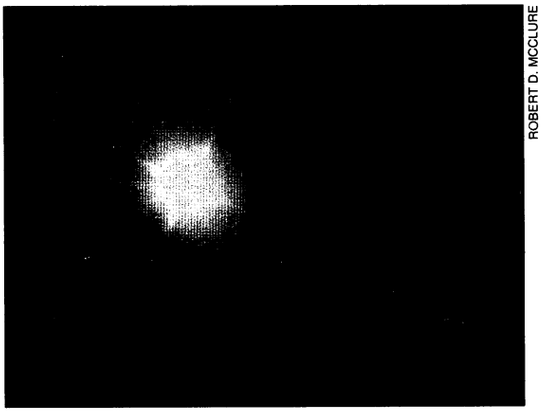
In Budget Crunch, FUSE Gets Trimmed

Astrophysicist George Sonneborn of the National Aeronautics and Space Administration's Goddard Space Flight Center sounded surprisingly upbeat last week, considering that NASA had just slashed the funding for his ultraviolet satellite project by more than half. Wesley Huntress, NASA's associate administrator for space science, had told Sonneborn and his colleagues at NASA-Goddard, Johns Hopkins University, and institutions in Canada and France that budget pressures are forcing the agency to "terminate" the Far Ultraviolet Spectroscopic Explorer (FUSE), a $250-million orbiting observatory that was due to fly by 2000. The agency did soften the blow, however, by offering FUSE a chance at resurrection as part of a much cheaper series of space science missions, with price tags of around $100 million. Sonneborn and his colleagues say they will seize the opportunity.

"We are now looking to redesign and restructure the program to do the most important science that FUSE was intended for," Sonneborn says. With a cheaper rocket, a lower orbit, a shorter life span, and less documentation, says FUSE participant Mark Perry of Johns Hopkins, it should be possible to shrink the price without serious compromises. "You put a lot in to get that last little bit out," he notes. If the redesign succeeds, the mission should yield many of the promised insights into the big bang and the structure of our galaxy, but it will no longer be a tempting target for NASA budget cutters.

Approved in 1989, FUSE was to have been the final and most expensive of an ongoing series of medium-sized space science missions known as the Explorer program, which has included the Cosmic Background Explorer and the International Ultraviolet Explorer. But as Huntress explained last week, NASA had planned the entire Explorer series during the early 1980s assuming 6% to 7% increases in its space science budget each year. "These anticipated augmentations have not materialized," he says. Something had to give.

Costing nearly twice as much as the next most expensive Explorer mission and still in the design stage, FUSE was the obvious place to cut, explains NASA spokesperson Donald Savage. But that's no reflection on its potential for science, says co-investigator Andrew Michalitsianos of NASA-Goddard. The far ultraviolet wavelengths FUSE was to detect are "one of the last unexplored regions of the spectrum," he notes, harboring clues to the makeup of the tenuous gases between the stars. And in 1990 a panel of astrophysicists led by John Bahcall of the Institute for Advanced Study in Princeton put FUSE high on its list of space science priorities.



ROBERT D. MCCLURE

Search light. By looking for absorption in the light of distant quasars like this one, known as 3C 273, FUSE would sample the makeup of interstellar gas.

Among its goals were to measure the temperature and composition of the interstellar medium by pointing spectroscopes toward distant stars, galaxies, and quasars and detecting wavelengths absorbed by the intervening gas. A key objective was to determine the ratio of ordinary hydrogen to the heavier isotope deuterium, which holds clues to the density and distribution of matter during the first minutes after the big bang, when those two isotopes formed. The Hubble Space Telescope has sampled this cosmic ratio in the region very close to our solar system. But because FUSE was designed to distinguish close-spaced absorption lines that blur together in Hubble spectra, it was expected to carry the measurements out to greater distances, says Sonneborn.

Researchers had also been counting on FUSE data to help explain a puzzling finding from earlier studies of our galaxy: the presence of patches of hot gas, reaching temperatures of a million degrees. The heat might be left over from the big bang, says Sonneborn, but the gas has had plenty of time to cool. "Why is it hot? Why is it still hot?" he asks.

NASA isn't asking FUSE scientists to give up on these questions—if they can address them within a budget less than half the size of the original project's. Technically, the old FUSE was part of the largest subset of Explorers, the Delta class, while the reborn FUSE mission would be part of a smaller class called the medium explorers, or MIDEX. Huntress predicts that the researchers can successfully trim the project down to size. But with 90 days to submit a revised plan and $150 million in costs to shed, Sonneborn and his colleagues will need all the optimism they can muster.

–Faye Flam