CELL BIOLOGY

Getting a Grip on G Protein Function in *C. elegans*

When it comes to the G proteins, cell biologists have amassed a great wealth of material. They have identified nearly 30 of these proteins, which serve as key relays in the pathways that transmit signals from hormones, neurotransmitters, and other cellular regulators from the cell membrane to the interior. And studies with cultured cells have enabled researchers to learn a great deal about the biochemistry of G proteins. But just as piling up material wealth doesn't always lead to happiness, the simple accumulation of G proteins doesn't automatically produce a better understanding of their biological functions. What many of them actually do in living organisms, for example, is still unknown.

Take the G protein known as $G\alpha_o$. Researchers suspect that it has an important role in the body, partly because it is extremely abundant in nerve tissue. Nevertheless, says biochemist Alfred Gilman of the University of Texas Southwestern Medical Center in Dallas, who shared last year's Nobel Prize in medicine for his G protein work, $G\alpha_o$ has been "a protein looking for a function." Now, two research teams, one led by Joshua Kaplan of Harvard Medical School and the other by Paul Sternberg and Melvin Simon of the California Institute of Technology and Ronald Plasterk of the Netherlands Cancer Institute in Amsterdam, may have remedied that situation, at least in the roundworm *Caenorhabditis elegans*.

In papers that appear on pages 1648 and 1652, they report genetic evidence that $G\alpha_o$ takes part in the signaling pathways that control several of the worm's behaviors, including locomotion, egg-laying, and male mating. The result poses one puzzle, because it fails to confirm that $G\alpha_o$ has a role in nervous system development, as researchers had suspected. But it's a big step toward solving others, says G protein expert Elliott Ross, also of Southwestern Medical Center. "It maps out part of a signaling pathway in an intact organism."

The work should provide a starting point for tracing out other components of the complete G protein signaling pathway in *C. elegans*, a favorite subject for geneticists because of its simplicity—adult worms have only about 1000 well-defined cells—and rapid life cycle. The components of the worm's pathway should, in turn, aid in identifying the participants in the comparable pathway of higher organisms, including mammals. As Simon, a longtime worker in the G protein field, points out, $G\alpha_o$ and the other G proteins of *C. elegans* "are very homologous to the proteins in vertebrates." The hope is that the other components of G protein pathways will show similar evolutionary conservation.

JANE MENDEL / HHMI

Highs and lows. Loss of $G\alpha_o$ produces hyperactive worms *(middle)*, while worms with too much $G\alpha_o$ activity are sluggish *(lower left)*. The top worm is normal.

For Kaplan, the G protein results are an unexpected bonus, because he did not set out to study G protein function at all. He originally wanted to follow up on clues from several labs, including that of Robert Horvitz at the Massachusetts Institute of Technology, that the neurotransmitter serotonin is an important regulator of *C. elegans* behavior. Kaplan himself has shown, for example, that worm mutants that lack serotonin are hyperactive. To try to figure out how serotonin exerts its effects, Kaplan set out to find additional genes needed for its action.

He began with two mutant strains of *C. elegans*, which had been identified by Michael Finney and Victor Ambrose while in the Horvitz lab. Both mutants had lost the ability to respond to serotonin, and Kaplan and his Harvard colleagues Laurent Ségalat and Daniel Elkes first confirmed that, as expected, they are hyperactive. The researchers then identified the gene at fault, which turned out to encode none other than $G\alpha_o$. Together, these findings suggest that the protein is needed for relaying serotonin signals to the cell interior.

Further evidence for that hypothesis came in additional experiments, including one in which the Harvard workers compared the behavioral effects of mutations in *goa-1* (the official name for the $G\alpha_o$ gene) to those caused by mutations that prevent production of serotonin itself. The result, Kaplan says, is "that we could get the same sort of modification of behavior" in the two types of mutants. In addition to being hyperactive, for example, both had sluggish digestion. And when the Harvard team used genetic engineering to increase expression of *goa-1*, they got the opposite effects: The worms' locomotion slowed and their digestion speeded up. "Basically, we showed that the dose of $G\alpha_o$ is a critical determinant regulating the behaviors," Kaplan says.

Meanwhile, Sternberg, Simon, and their colleagues were coming to similar conclusions from the opposite direction. They started by homing in directly on *goa-1*, using genetic engineering methods to knock out the gene. This, they found, produced behavioral defects such as hyperactivity and premature egg-laying. Again, just the opposite happened when they introduced a mutation into *goa-1* that increased the activity of $G\alpha_o$. The Caltech group also noted that both types of gene alteration impaired the mating abilities of male worms, indicating that normal $G\alpha_o$ function is also needed for that behavior.

$G\alpha_o$'s role in these behaviors was not surprising, Sternberg says, because both groups' work showed that $G\alpha_o$ is widely expressed in nerve and muscle tissue. Still, several researchers familiar with G proteins and *C. elegans* were startled by one finding. Because $G\alpha_o$ is a prominent component of the "growth cones" located at the tips of developing neurons, its signaling function was thought to be important for the formation of normal neuronal connections. But in the new work, mutating the gene encoding $G\alpha_o$ did not produce major disruptions of neuronal wiring. "The most surprising thing is that the phenotypes [alterations caused by the mutations] are quite subtle," says *C. elegans* researcher Cori Bargmann of the University of California, San Francisco. "If people had taken a guess, they would have expected more severe phenotypes."

Why *goa-1* mutations didn't produce obvious disruptions of neuronal development remains unclear. One possibility, Sternberg suggests, is redundancy—the ability of some other G protein to take over in $G\alpha_o$'s absence. Or $G\alpha_o$ may simply not be as important in growth cone function as thought.

Even though the mutants pose a puzzle, they are a good start toward mapping the rest of the $G\alpha_o$ signaling pathway. "What the mutants do is give people behavioral systems where they can look for the next component in the path. That's not easy to do with [cultured] cells," says Ross. Particularly helpful, Bargmann notes, should be the "gain-of-function" mutants both teams have created, in which $G\alpha_o$ production is excessive. Gain-of-function mutants are "a powerful tool," she notes. "It's really nice" that they have such mutants.

The researchers plan to take advantage of their tool by inducing new mutations in the gain-of-function mutants, then screening for individuals whose behavior has been corrected by the mutations. The affected genes are likely to be acting downstream of $G\alpha_o$, because their inactivation blocks the excessive signaling. If all goes well, those genes could in turn be used to produce probes for tracking down comparable genes in mammalian cells.

"The interesting thing is that they can go beyond C. *elegans* to dissect the entire pathway," says Horvitz. Completing the work will probably take other approaches besides genetic studies in C. *elegans*, among them developing G protein knockouts in mice—an effort that is already under way in several labs—and continuing to study G proteins in cultured cells. In the end, though, G protein researchers should finally get some satisfaction from their growing wealth.

–Jean Marx

GEOPHYSICS

Shock Test Squeezes Core Temperature

Heat makes the world go around, or at least the world of geophysics. Heat drives the magnetic dynamo of Earth's liquid-iron core, it slowly churns the rocky mantle, and it drives plate tectonics and all its attendant phenomena from volcanoes to earthquakes to mountain building. But just how much heat Earth has in its deep interior and how it flows outward to shape the surface is a major unknown in geophysics—and one of its biggest bones of contention. One arena for the conflict is a new generation of lab experiments to probe the melting point of iron at the extreme pressures found in Earth's core. In 1987, these experiments came up with a temperature that was much higher than expected, and researchers have been working ever since to confirm—and explain—the startling result.

They have faced a big problem in trying to recreate the conditions in Earth's core in the lab, but refinements to the techniques used in 1987 have recently enabled several groups of researchers to come up with some new, independent estimates. To their relief, the predicted temperature of Earth's core has dropped down a notch. "No one would stick their neck out for the high temperatures [of 1987]," says Jean-Paul Poirier of the Institute of Physics of the Globe in Paris. But even these new results, from experimental work by Thomas Ahrens and his colleagues at the California Institute of Technology (Caltech) in Pasadena, still suggest an uncomfortably hot inner Earth.

The focus of the work is the temperature at the boundary between the solid-iron inner core and the molten outer core 5100 kilometers beneath the surface. This is the benchmark temperature of Earth's interior. The melting temperature of any material—the temperature at which solid and liquid coexist—has a fixed value for a given pressure, and as geophysicists know the pressure at the inner core/outer core boundary from the weight of material above it, by measuring the melting point of iron at that pressure they can determine the temperature at the boundary.

A landmark attempt to take the temperature of the inner core was reported in a 1987 *Science* paper describing two types of cutting-edge measurements of the melting point of iron. Quentin Williams of the University of California, Santa Cruz, then a student at the University of California, Berkeley, and Raymond Jeanloz of Berkeley reported melting-point measurements up to pressures of 100 gigapascals (GPa)—1 million times atmospheric pressure—created by squeezing a speck of iron between two diamonds while laser-heating it to 4000 K. The pressures in their diamond-anvil cell, at least a factor of 5 higher than in any similar iron-melting experiment, still fell far short of the 330 GPa in the Earth's inner core, however. To continue to higher pressures, Jay Bass of the University of Illinois, Urbana-Champaign, then visiting Caltech, Bob Svendsen of Caltech, and Ahrens melted iron between 200 and 300 GPa in high-pressure shock experiments in which the iron sample is blasted by a flat metallic "bullet" shot from a gas-powered gun at up to 7 kilometers per second.

THOMAS J. AHRENS/CALTECH SHOCK WAVE LABORATORY

Big guns. Shock waves produced in high-speed guns can melt iron under conditions of Earth's core.

The combined diamond-anvil and shock-melting results traced iron's rising melting point under increasing pressure. When these data were extrapolated a bit to the pressure of the inner core, they gave the stunningly high temperature of 7600 K. Even allowing for impurities in the core that might lower the melting point by 1000 K, this result was way above the textbook temperature for the inner core of around 4000 K.

Jeanloz, for one, was not fazed. He began work on a new view of Earth's interior, proposing a model in which the core still carries a hefty load of heat from its formation 4.5 billion years ago and contributes more than 20% of the heat flowing to the surface. Jeanloz's "hot Earth" model included a hot lower mantle to blanket the core and prevent heat from reaching the relatively cold upper mantle. This would also require a lower mantle rich in heavy elements to prevent mixing and enforce the stratification.

Because the hot Earth model specifies so many new characteristics of Earth's tectonic heat engine, most researchers remained skeptical. Among those was Reinhard Boehler of the Max Planck Institute for Chemistry in Mainz, Germany. He has been melting iron in a diamond-anvil cell since 1986, gradually making improvements on the pioneering work of Williams and Jeanloz and consistently coming up with figures 1000 K below their melting point at 100 GPa.

Why the results of the two diamond-anvil labs disagree is not yet understood, but Ahrens and George Chen of Caltech have modified the shock experiment so it can be used as a check on the lower pressure diamond-anvil results. They found that by preheating the iron sample, they could get it to melt with a lighter shock at pressures below 100 GPa. "We've done an independent set of experiments and we're very, very close to Boehler's data" below 100 GPa, says Ahrens.

Prompted by new doubts about his original shock experiments, Ahrens took another look at them as well. He and Kathleen Gallagher of Caltech began to look at the aluminum oxide casing, which holds the iron at high pressures during the critical few hundred nanoseconds of an experiment and acts as a window for the radiant energy emitted by the iron that tells the experimenter its temperature. If that window material were conducting less heat out of the iron than had been calculated from theory, the temperatures attributed to melting points would be too high.

Gallagher and Ahrens indeed found that the aluminum oxide was not behaving as their theoretical calculations had predicted. "That changes our ideas—our apparently mistaken ideas—about the behavior of mate-