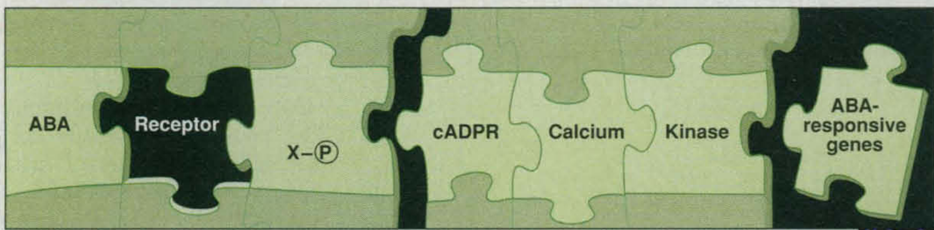
Plants Decode a Universal Signal

The molecule cyclic ADP–ribose is placed firmly in a pathway that helps plants respond to stress, paving the way toward a better understanding of this messenger's role in all cells

When winter's bitter tongue lashes south of the frost line, Florida vacationers can always head to a Caribbean island. Plants don't have that luxury. Palm trees and citrus groves have to tough it out right where they are, by activating a stress response that makes them more tolerant of cold or drought. But orange trees and tourists aren't as different as they seem. A team led by plant molecular biologist Nam-Hai Chua of Rockefeller University in New York City has identified a signaling molecule that helps trigger plant stress responses to low temperatures and drought. In so doing, the researchers may have opened a window on how animal cells regulate all sorts of things, from heartbeat to insulin secretion.



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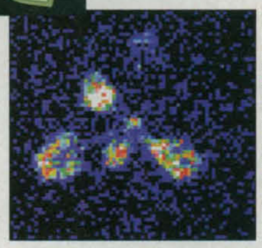

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Fitting the pieces. Working through an as yet unidentified receptor, the stress hormone (ABA) uses cyclic ADP–ribose to release Ca, activate a kinase enzyme, and turn on certain genes, as evidenced by luminescence in *Arabidopsis (right).*

Plant biologists already knew that the stress responses are turned on when a specific plant hormone triggers a surge in calcium ions inside the cell. They did not know, however, exactly how the hormone does this. On page 2126, the Chua group now reports that a molecule called cyclic ADP–ribose is what relays the hormone's signal to the stores of calcium in the interior.

Researchers already suspected that the same molecule helps to control calcium in animal cells. Cyclic ADP–ribose, says Anthony Galione, a pharmacologist at the Oxford University in the United Kingdom, "looks like a ubiquitous messenger across the plant and animal kingdoms." The Chua group's finding will help researchers pin down in animals how other signal molecules interact with cyclic ADP–ribose to control calcium. "It's the first definitive evidence of a significant role for cyclic ADP–ribose," says Dale Sanders, a cell biologist at the University of York in the U.K.

Besides tracing what may be a striking example of a single molecular mechanism conserved across the tree of life, the discovery could have practical payoffs. In plants, it might open the way to producing crops that resist stresses better, perhaps by engineering them to respond more quickly in tough conditions. And in humans, it could help investigators understand disorders ranging from heart arrhythmias to diabetes.

Although the focus of the current work is plants, animal cells—sea-urchin eggs—provided the first evidence that ADP-ribose regulates calcium. Almost 10 years ago, Chua's collaborator Hon Cheung Lee, a cell physiologist at the University of Minnesota, Minneapolis, was studying the wave of calcium ions that touches off the embryo's early development. At the time, cell biologists thought that a compound called inositol trisphosphate (IP_3) was the cell's internal calcium dispatcher. But Lee noticed that adding a substance called NAD could also stimulate a calcium wave. The NAD did not seem to be acting by triggering the IP_3 signal, because the calcium buildup was slower than the rise seen when he added IP_3, and it occurred even in cells that no longer responded to IP_3. Puzzled, Lee hypothesized that something else—presumably a breakdown product of NAD—was freeing calcium from its internal stores. In 1989, he found that "something else": cyclic ADP–ribose.

At first, other cell biologists were skeptical. But time has proved that cyclic ADP–ribose does regulate calcium. "Since then, about 40 different cell systems from 14 different species—ranging from plants and protozoa to mammals—have been shown to be responsive to [cyclic] ADP–ribose," says Lee. Yet no one had been able to place this calcium regulator into a specific pathway until now.

Knowing that calcium ions are involved in plants' stress responses, Chua decided in 1995 to find out whether cyclic ADP–ribose was behind the increases. The team approached the problem by injecting the stems of tomato seedlings with either of two genes that respond to stress, or with a third gene that is activated by light. All three were attached to marker genes that enabled the researchers to monitor them when activated. As expected, injections of the plant stress hormone abscisic acid (ABA) turned on the two stress-responsive genes, *rd29A* and *kin2*, while the light-responsive gene remained off. Cyclic ADP–ribose by itself, without the hormone, could also activate *rd29A* and *kin2*. But inhibitors of cyclic ADP–ribose or of calcium-ion release prevented the genes from being turned on, even when the stress hormone was present. "We show that cyclic ADP–ribose mediates the [stress response] signal," Chua concludes.

The researchers also monitored *kin2* expression in the tiny plant, *Arabidopsis thaliana.* To trace the gene's activity, they linked the DNA sequence that regulates it to *luciferase*, the gene responsible for the firefly's glow, so that the firefly gene would be turned on whenever *kin2* was active. They then introduced the hybrid gene into *Arabidopsis*. After adding ABA, the researchers watched the luminescence of the plant tissue rise to a peak after about 3 hours. The rise came shortly after increases in both cyclic ADP–ribose concentrations and calcium ions. "The [researchers] show a causal relationship," comments Dierk Scheel, a plant molecular biologist at the Institute for Plant Biochemistry in Halle, Germany. "These experiments are wonderful."

At the University of York, Sanders has unpublished evidence that cyclic ADP–ribose triggers another stress pathway: the closing of the microscopic openings on the leaf surface known as stomata in response to drought. Working with Alister Hetherington at Lancaster University in the U.K., Sanders's team coinjected cyclic ADP–ribose and a dye that tracks calcium release into the guard cells which surround the stomata and control their size. "We showed that not only did the guard cells close, but that [the closure] was preceded by an increase in calcium," notes Sanders. The researchers also demonstrated that they could block both responses of the guard cells, even in the presence of stress hormone, by adding a cyclic ADP–ribose inhibitor.

In animals, researchers have not yet worked out specific pathways to the extent the Chua team has in plants, but they are making progress. Over the past 2 years, Galione's team has shown that cyclic ADP–ribose helps control one calcium-dependent process: cardiac muscle contraction. Working with heart muscle cells isolated from the guinea pig, he has found that too little of the messenger can lead to inadequate contractions, while too much can make the heart beat out of control.

Other work has implicated cyclic ADP–ribose in glucose-stimulated release of insulin from the pancreas. "There is the intriguing possibility that defective signaling could be one way diabetes arises," says Galione. This messenger also seems to be part of signal-transduction pathways in the reproductive and immune systems, and in the regulation of smooth muscle cells by thyroid hormone.

To be sure, calcium levels in cells respond to many other signals besides cyclic ADP–ribose. "There are actually multiple mechanisms for the releasing of calcium," stresses Galione. In nature, these multiple mechanisms may cause calcium to be released from different storage sites and in different patterns, generating a calcium "signature" unique to each signaling pathway. Galione and Chua suggest that this may be how the cell can keep its signals straight even though the same ion, calcium, is involved in many different signaling pathways.

Even for the plant stress pathways that depend on cyclic ADP–ribose, many details remain to be ironed out. Researchers do not yet know, for example, how it leads to the activation of ADP–ribosyl cyclase, the enzyme that generates cyclic ADP–ribose. However, Chua's work provides a clue. He has found evidence that the activation step requires the addition of a phosphate group, possibly to the cyclase.

Chua now hopes to pin down the other details of this pathway. "It has tremendous agricultural implications," because it could be a key to designing hardier crops, he explains. But "in order to design methods to engineer plants to resist drought, you need to know about the signal to be transmitted."

Like Chua, Galione is working hard to identify all the components in the pathways between, for instance, the signal to contract and the heart contraction. Others are trying to understand the steps between glucose's arrival at a pancreatic β-cell and the release of insulin. And because molecular biologists are now able to make molecules that mimic or block cyclic ADP–ribose activity, Galione expects rapid progress. "We should really be able to nail these pathways down," he predicts—and explore an unexpected similarity between plants and people.

–Elizabeth Pennisi

ASTRONOMY

Black Hole Lurks in Miniature Quasar

Everything about quasars and active galaxies is outsized. These brilliant objects lie millions to billions of light-years from Earth and, at their centers, may harbor black holes with masses millions of times that of the sun. But astronomers are getting a closeup view of quasar behavior in a scaled-down model—a so-called microquasar in our own galaxy.

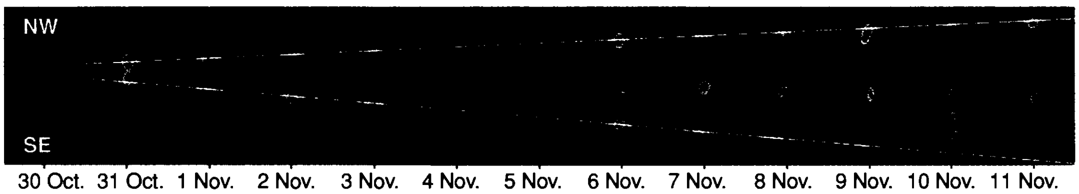
Like its full-sized relatives, the microquasar is thought to contain a black hole—in this case just a few times more massive than our sun—and it shows similar violent behavior, sometimes ejecting high-speed streams of ultrahot gas. Because of the microquasar's small size, its jets and flares develop and die out much faster than those of larger quasars, in minutes or hours rather than millions of years. This fast-forward view of quasar behavior is yielding new clues to how quasars work, including new evidence for the reality of the black holes at their centers. As Michael Garcia of the Harvard-Smithsonian Center for Astrophysics puts it, "You can actually see things happen in a minute" in the microquasar. "You can't see things like that happen in a [full-sized] quasar."

A team led by Felix Mirabel of France's Atomic Energy Commission at Saclay and Luis Rodriguez of the Institute of Astronomy in Morelia, Michoacán, Mexico, discovered the quasarlike behavior 3 years ago. With the Very Large Array radiotelescope in New Mexico, they observed jets of matter coming from a known x-ray source called GRS 1915, just 40,000 light-years from Earth. After other observers spotted radio outbursts from the same source, astronomers concluded they were seeing a system containing a small black hole—probably the legacy of a collapsed star—surrounded by an "accretion disk" of gas and dust dragged from a companion star. The interplay between the accretion disk and the black hole somehow powers the outbursts, just as in a full-sized quasar.

Last month, Ralph Spencer of the University of Manchester in the United Kingdom and several colleagues followed the evolution of GRS 1915's jets for 2 weeks using MERLIN, a set of six electronically linked radiotelescopes spaced across England. They found, reports Spencer's co-worker Rob Fender of the University of Amsterdam, that "the jets appear to be generated in less than a day, and we see the material speeding out over something like 2 weeks." Mirabel says that he observed the same event on 31 October with the Very Long Baseline Interferometer, which combines telescopes from Hawaii to the Virgin Islands. Once the data are processed, he says, "we will be able to see the blobs with a resolution equivalent to the size of the solar system."

By studying these fast-changing jets, astrophysicists are already gaining clues to their origin. In a paper scheduled to appear in the February issue of *Astronomy and Astrophysics*, Mirabel and his colleagues report that they traced jet formation by monitoring the microquasar at many different wavelengths—x-ray, infrared, and radio—sensitive to different parts of the accretion disk and jets. First, he says, "x-radiation from the inner accretion disk disappeared," presumably because superhot material fell into the presumed black hole. Then a new x-ray burst indicated that the inner disk was refilling with new material, and a dozen minutes later the first hints of a jet appeared. The findings, he says, support a picture in which the spinning black hole ejects the jets whenever material is building up in the inner disk.

The abrupt cutoff of the x-rays at the beginning of this process—often in a matter of seconds—is a strong hint that a black hole really is present, says Mirabel. The cutoff would not be so sudden if the material landed on the surface of a star, he says. Mirabel argues that the only explanation is that the globules of matter must suddenly be disappearing beyond the "event horizon" of the black hole. Garcia calls that picture "a very good model," although not "absolute direct evidence" for a black hole.



SPENCER *et al.*

Outburst. Probably driven by the spin of a central black hole, blobs of ionized gas fly outward from the microquasar.

Still, says Martin Rees of Cambridge University in the U.K., further study of the microquasar is sure to cast more light on its shadowy heart and those of full-sized quasars: "In the next year or two, we [may] actually learn something about the nature of black holes."

–Alexander Hellemans

Alexander Hellemans is a writer in Naples, Italy.