

AUGUST 22, 2007

Reading the Leaves for Clues to Origin of Rare Epilepsy

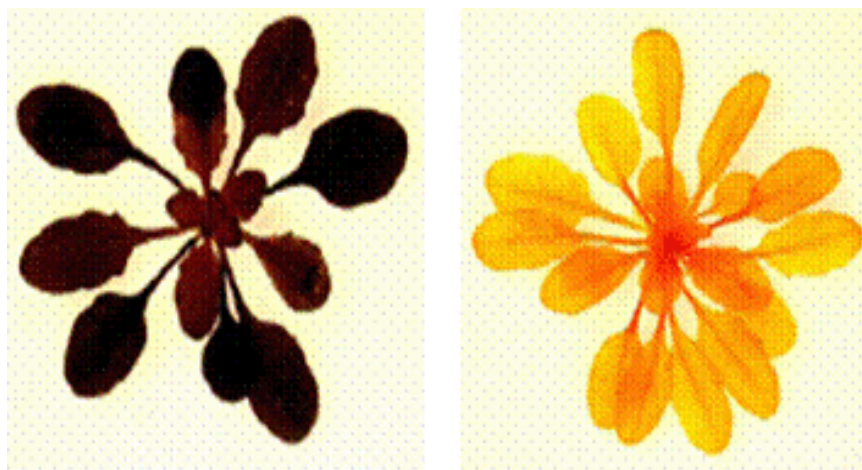


Image Title: Researchers monitored carbohydrate production in *Arabidopsis* plants by using a simple iodine stain. During the day, stained plants remained black (left) due to high levels of carbohydrates in the chloroplasts. At night, when the plant had metabolized the carbohydrates, the leaves turned white (right). - Courtesy of Jack E. Dixon

For a century, scientists have known that Lafora disease is a progressive and deadly form of epilepsy caused by a buildup of carbohydrates in the brain. Although this type of epilepsy has been well described in patients, researchers have been at a loss to explain precisely why - on a molecular level - neurons begin to accumulate toxic amounts of carbohydrate.

A hint about the nature of this malfunction is reported in the July 2007 issue of the *Journal of Cellular Biology*. A research team led by Jack Dixon, professor and Dean of Scientific Affairs at the University of California San Diego (UCSD), has shown that humans, plants and protozoa all seem to rely on the same basic cellular machinery to solve the problem of carbohydrate buildup.

The paper published by Dixon, who is vice president and chief scientific officer at Howard Hughes Medical Institute, and his colleagues at UCSD and

the Salk Institute for Biological Studies outlines the common mechanism that humans and other organisms use to purge excess carbohydrates, and how this information could help develop treatment for Lafora. While Dixon suspects that carbohydrate buildup can have detrimental effects on other species as well, its harmful effects on humans are terribly clear.

"We realize that this is a very hard problem, but we like hard problems, and we're not afraid of tackling those at all."

- Jack E. Dixon

Children with Lafora disease have no symptoms until an initial epileptic seizure around age 10. The children later experience frequent seizures and ultimately develop dementia. Patients rarely survive a decade past their diagnosis.

Lafora disease is caused by a mutation in the gene that codes for the enzyme laforin, which belongs to a group of phosphate-removing proteins called phosphatases. It is the only known phosphatase in vertebrates that binds to carbohydrates, using a molecular "zip code" called a carbohydrate-binding domain.

Normally, neurons contain relatively few carbohydrates. For people who cannot produce laforin, however, carbohydrates accumulate in the brain and become toxic. Scientists still do not know why removing a phosphate group prevents this deadly buildup. In fact, scientists have known little else about laforin for quite some time.

Looking Through the Trash

New information about laforin arose from a set of serendipitous clues. Dixon's lab probably wouldn't have looked for laforin in other organisms had it not been for Matthew Gentry, one of Dixon's postdoctoral fellows. Earlier in his academic career, Gentry worked with *Toxoplasmodium gondii*, a single-celled organism carried in cats that causes toxoplasmosis in humans. The disease is potentially fatal to human fetuses and adults with weak immune systems. Recently, Gentry stumbled upon pictures of the protozoa with deposits of a carbohydrate called floridean starch. *T. gondii* burns floridean starch when active and stores it when inactive. He noticed that images of inactive *T. gondii* cells looked like neurons of patients with Lafora.

Gentry wondered if the protozoan produced a carbohydrate-binding enzyme like laforin to prevent excess starch buildup. After looking in vain through the online databases at the National Center for Biotechnology Information

(NCBI) for the *T. gondii* genome, Gentry eventually turned to Google.

Gentry then began what Dixon jokingly calls, “looking through the trash.” The “trash” refers to the mass of As, Cs, Gs and Ts with unknown functions in sequenced but unannotated genomes. Many of these are on the internet—so searching for a snippet of *T. gondii* genetic code that looked like laforin meant scrolling down scores of websites. “I must have looked at about 170 different genomes and checked every phosphatase to see if it had a carbohydrate-binding domain,” Gentry says. “It wasn’t a lot of fun.”

Gentry ultimately found a carbohydrate-binding phosphatase in the *T. gondii* genome that looked exactly like laforin. Encouraged by this success, Gentry, Carolyn Worby, a research scientist in Dixon's lab, and Seema Mattoo, a microbiologist and postdoctoral fellow in the lab, found laforin in several other unicellular eukaryotes. Since mutations of laforin cause Lafora disease in humans, Dixon hypothesizes, “mutations of laforin in these organisms probably limit their ability to function as well.”

To further probe the universality of the enzyme, the researchers turned to the kingdom full of the best carbohydrate processors on the planet—plants.

The Elegant Experiment

Dixon culled through literature on flora to find a carbohydrate-binding enzyme. He eventually discovered the plant counterpart to laforin in a 2006 paper written by Totte Niittyla in Alison Smith's lab at the John Innes Centre in Norwich, UK.

Niittyla identified a mutated enzyme in *Arabidopsis*, a member of the mustard family. Scientists use *Arabidopsis* for genetic experiments because they can easily remove, mutate, and replace its genes. The mutant plant that Niittyla studied was carbohydrate-heavy, and had a mutated gene called *SEX4* (or starch excess 4). *SEX4* looked different than laforin, but contained a “zip code” that indicated it was targeted to the chloroplast—an area of high carbohydrate production.

Dixon had a hunch that *SEX4* prevented carbohydrate buildup in plants, much like laforin did in humans. To pursue his hypothesis, Gentry and Robert Downen, a graduate student in Dixon's lab working on *Arabidopsis* with the assistance of plant biologist Joseph Ecker from The Salk Institute, monitored carbohydrate production of *Arabidopsis* plants using a simple iodine stain. During the day, stained plants remained black due to high levels of carbohydrates in the chloroplasts. At night, when the plant had metabolized the carbohydrates, the leaves turned white. Plants with mutations in the *SEX4* gene developed the plant equivalent of Lafora disease -- they held onto their carbs and remained dark-colored at night.

Dixon acknowledges that mutated, high carbohydrate content plants could appeal to people interested in producing more efficient ethanol for fuel. “But from our point of view,” he says, “it's a very lovely example of how nature reinvents things that are required.”

Researchers in Dixon's lab pushed the extent of the versatility of these enzymes. They decided to test if the human gene for laforin could function in plants. Dixon says, “We bet on this in the lab—about whether it would work or not—and we were split about 50/50.”

The results, however, were literally black and white. As long as both enzymes were connected to the correct molecular zip code, they removed carbohydrates equally well. Laforin, attached to a chloroplast-targeting domain, kept carbs in check in plants with SEX4 mutations, turning leaves white by nightfall. Laforin and SEX4 were ineffective, however, without the domain. In both of those trials, *Arabidopsis* leaves stayed black and full of carbs.

The results speak to Dixon's belief in a trans-kingdom approach to science. Gentry comments, “His ability to take information from one organism and apply it to another coupled with his success in bringing together researchers with diverse skill sets really speeds up how quickly you can learn about biological processes. I think that's given us great insight into Lafora disease.”

The carbohydrate-removal mechanism in other species could provide clues for treatment of Lafora in humans. Despite the value of new information on laforin, reintroducing the gene to the brain presents complicated obstacles. Still, Dixon is hopeful: “We realize that this is a very hard problem, but we like hard problems, and we're not afraid of tackling those at all.”