MEETING PACIFICHEM 2000

# Pacific Chemists Throw Switches, Strike at Disease

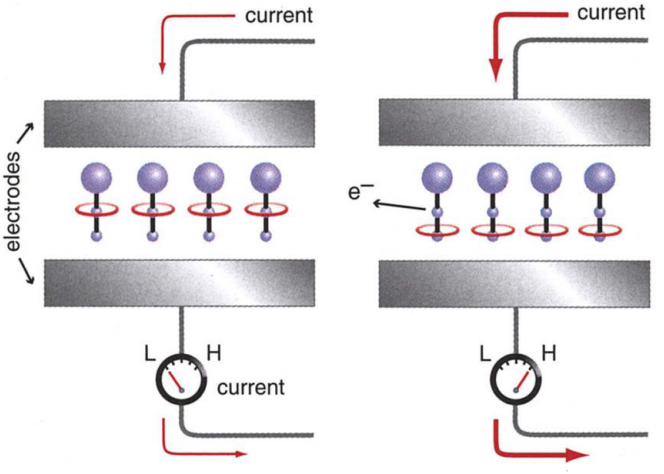
**HONOLULU, HAWAII—Once every 5 years, chemists from North America, Japan, New Zealand, and Australia come together for the International Chemical Congress of Pacific Basin Societies. At last month's meeting, over 10,000 researchers discussed topics that included a new molecular electronic switch and new hope for fighting diabetes and Alzheimer's disease.**

## Organic Molecular Switch

In the drive to create ever-smaller computer chip components, making devices with molecules that switch on and off like transistors is all the rage. So far, such devices have had problems: Either they've required freezing temperatures to operate, or they haven't passed enough electrical current to be useful. But now a team has come up with new molecule-based switches that work at room temperature while passing large currents. The new molecules are already giving the nascent field of molecular computing a lift. "I think this is pioneering stuff," says chemist Peter Stang of the University of Utah in Salt Lake City.

In recent months, researchers have demonstrated several types of molecular switches. One team at the University of California, Los Angeles, used a set of molecules called catenanes, made from pairs of intertwined molecular rings. By changing electrical voltages applied to electrodes sandwiching the catenanes, the team showed that they could alter the number of electrons on the rings. This caused the rings to rotate around one another, which altered the ability of current to flow through the catenanes from one electrode to the other. But in constructing the devices, the researchers had to layer the rings atop hydrocarbon molecules. This setup placed the rings closer to one electrode than the other and made it difficult for charges to move through the mix.

To improve the design, the same team, led by chemist Fraser Stoddart and postdoc Julie Perkins, constructed a new class of switching molecules. Called pseudorotaxanes, they are shaped like molecular lollipops. Like the catenanes, the pseudorotaxanes are sandwiched between a pair of electrodes. Positively charged ring-shaped compounds nestle close to an electron-donating portion of the lollipop stem. When an electrical potential is applied between the electrodes, it yanks electrons off the stem area and spurs the ring-shaped compounds to jump to another spot. This jump lowers the electrical resistance of the molecular switch and allows current to flow more easily between the surrounding electrodes, the researchers reported.




**Electrifying.** When ring-shaped compounds slide down their stems, lollipop-like pseudorotaxane molecules become much more conductive.

In addition, the physical structure of the new switch design fosters current flow. Like the catenanes, the pseudorotaxanes still need hydrocarbons to help them assemble properly between the electrodes. But, in this case, the lollipops and hydrocarbons sit side by side, allowing the lollipops to be centered between the two electrodes and prompting nearly 100 times more current to flow between the electrodes.

Stang notes that the pseudorotaxanes aren't ready to compete with Pentium chips just yet, as they tend to break down after a week or so of use. Stoddart says he believes that may be due in part to the fact that the rings can slide off the ends of the lollipops. His team is already at work on new versions of the molecules with bulky groups on both ends to prevent the rings from sliding off.

## A Key to Diabetes?

Geneticists have been toiling for years to identify the genes involved in adult-onset diabetes, in which cells fail to absorb glucose from the blood. The condition arises either because cells become unresponsive to insulin, the hormone that tells them to take up and store glucose, or because specialized sensor cells in the pancreas fail to detect glucose that should trigger the release of insulin. At the Honolulu meeting, Harvard University chemists reported the first evidence that both problems may stem from a single molecular source, a protein that appears to be the key to sensing nutrient levels in cells.

The Harvard team—led by chemical biologist Stuart Schreiber—described a combination of new and old work that showed how a small molecule called rapamycin that blocks this protein can both make cells unresponsive to insulin and affect their ability to properly sense glucose and other nutrients. So far, the team has studied nutrient sensing only in yeast, a model organism for understanding how cells sense nutrients. Still, the new evidence "is really quite remarkable," says Gerry Crabtree, a cell-signaling expert at Stanford University.

If the studies with rapamycin, an immunosuppressive drug given to organ transplant patients, show that it also affects nutrient sensing in human β islet cells of the pancreas, it could point to a common cause for both pathways to the disease. Crabtree, for one, believes there's a good chance that will happen, because yeast and humans have similar nutrient-sensing genes and proteins. "I think it has a reasonable chance of working out," he says.

If so, it could pave the way for new therapies to stop a condition that affects millions of people worldwide. Adult-onset diabetes commonly afflicts people who are overweight and sedentary. Left unchecked it can damage the heart, kidneys, nerves, blood vessels, and lead to blindness. In trying to understand the disease, researchers have traditionally focused on cells' progressive insensitivity to insulin. But the numerous genes fingered as possible culprits account for only about 10% of the cases.

The Harvard team—which included graduate students Alykhan Shamji and Finny Kuruvilla—didn't set out to find the other 90%. Rather, they decided to study the role of a protein dubbed FRAP that their laboratory had discovered years earlier. Schreiber and his colleagues knew that FRAP—and its twin found in yeast, called Tor—are signaling proteins involved in sensing and re-

ILLUSTRATION: J. MOGLIA

sponse to cellular stress. They also knew that rapamycin blocks FRAP and Tor. The chemists wanted to see what effect blocking these proteins has on the expression of other genes in the cell. To find out, they turned to the increasingly popular gene-chip technology, in this case a version designed to study yeast. The chips contain DNA snippets from all 6200 genes in yeast and are designed to reveal the activity of each gene, whether dormant or in hyperdrive. By comparing gene activity profiles of untreated cells with those of cells given rapamycin, the Harvard team teased out the effect of the drug on yeast's genetic machinery.

"There was a big surprise," says Schreiber. In response to rapamycin, yeast cells ramp up the production of genes involved in respiration and the production of the chemical fuel ATP, among other things. "Clearly these cells are trying to generate energy," says Schreiber. The surprising aspect was that these cells were sitting in a nutrient bath rich with glucose, their primary energy source. But they weren't using it. Instead, the yeast cells were turning on genes to process ethanol—their backup energy source—as well as proline and urea, backup sources of nitrogen. Indeed, the researchers found that their gene expression patterns looked just like those of yeast cells deprived of their primary nutrients.

"These [rapamycin-treated] cells are swimming in a sea of glucose. Yet they think they are starving," Schreiber says. From this he concludes that the drug has indeed blocked the cells' nutrient sensor, Tor.

That condition, Schreiber notes, is reminiscent of diabetes, in which cells bathed in insulin no longer respond to its message to absorb glucose. In fact, human patients who take rapamycin as an immunosuppressant commonly develop a diabetic-like condition as a side effect of the drug. Moreover, Schreiber says, previous work has shown that when cells that are normally responsive to insulin are given rapamycin, they become insulin resistant.

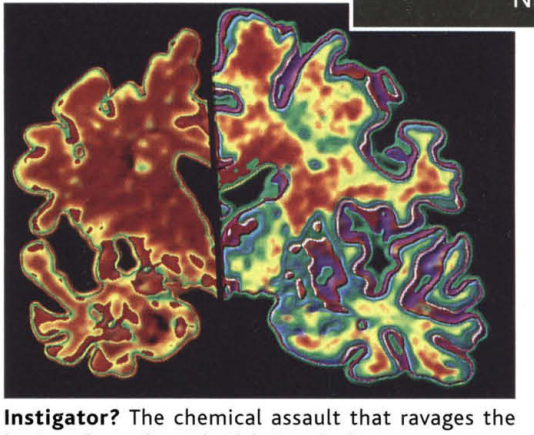
The group is currently looking at the response of pancreatic islet cells to rapamycin. If the drug also appears to trick these cells into thinking they are in a starvation environment, it would suggest that FRAP—Tor's mammalian counterpart—is indeed the master switch regulating cells' response to nutrients. For now that remains a big if. But if it pans out, it could give diabetes researchers new hope for finding a molecular keystone at the center of the disease.

## Alzheimer's Suspect

For researchers working to stop Alzheimer's disease, the chief molecular suspect has long been clear: a protein fragment called β amyloid that builds up in the brains of Alzheimer's sufferers. This presumably leads to the brain neuron loss underlying the disease, but pinning down just how β amyloid damages nerve cells has been hard. Some researchers suspect that at least part of its neurotoxicity is due to its promoting the creation of free radicals, reactive chemicals that can kill cells by tearing apart their proteins and fatty membranes. Just how β amyloid might foster free radical production has remained a mystery. But at the Pacifichem meeting, Kentucky researchers reported results linking free radical generation to a particular amino acid in β amyloid, and also showed that antioxidants such as vitamin E counter the damage.

The new evidence "is important," says William Klunk, who directs the Alzheimer's research program at the University of Pittsburgh Medical School in Pennsylvania. It lends heft to the notion that free radical damage could kill neurons around the β amyloid deposits in the brain. Other processes may also promote damage, Klunk stresses. But if the free radical mechanism holds up, it suggests that antioxidants targeted to cross easily into the brain and bind to β amyloid could help stave off damage from the disease. "That's an interesting hypothesis and a testable one," says Zaven Khachaturian, an Alzheimer's expert and consultant in Potomac, Maryland.

The notion that free radicals generated by β amyloid may play a role in Alzheimer's gained initial support in 1994 when Alan Butterfield and his colleagues at the University of Kentucky, Lexington, reported that the peptide produces free radicals in the test tube. Later, they and other researchers showed that reactions involving the peptide could form free radicals capable of setting off a chain of damaging reactions between lipid molecules in cell membranes.




**Instigator?** The chemical assault that ravages the brains of people with Alzheimer's disease may start with the amino acid methionine.

That still didn't pinpoint what it was in β amyloid that set the ball rolling in the first place. But Butterfield, a chemist, had a guess when he took a look at the peptide's amino acid sequence. Most of the 42 amino acids that make up β amyloid are fairly unreactive. But number 35, methionine, is a compound known as a thioether, a good free radical generator. To test whether methionine is indeed the culprit, Butterfield and his colleagues synthesized both normal β amyloid and a version that swapped a synthetic amino acid called norleucine for the methionine at position 35. Norleucine differs from methionine by a single atom, a carbon in place of a sulfur. When the researchers added the two versions of β amyloid to separate cultures of rat neurons, they found that the normal version of the peptide generated free radicals and killed the neurons, while the modified version had no such effect. When they then added vitamin E to the culture with the normal β amyloid, the antioxidant prevented free radical formation and cell death.

But that wasn't all. Butterfield's team went on to test their modified β amyloid in a living organism, the worm *Caenorhabditis elegans*. In this experiment, the researchers replaced methionine with cysteine, a natural amino acid. In collaboration with Christopher Link of the University of Colorado, Boulder, they genetically modified worms so that they made either normal human β amyloid or the cysteine-containing version. They found that the worms making the normal β amyloid generated free radicals that damaged their muscle proteins. Those making the cysteine version, however, had no damage and produced the same amounts of free radicals as did controls that received an empty gene-delivery vehicle.

Taken together, Butterfield says, the new results suggest that β amyloid's methionine amino acid is the primary culprit for generating free radicals that can go on to kill nerve cells. If this free radical damage winds up being the chief cause of the disease, it suggests that antioxidants may stem the tide. Early trials with high doses of vitamin E in humans have proven only moderately successful. But Butterfield notes that vitamin E isn't very efficient at crossing the blood-brain barrier and doesn't preferentially seek out sites with β amyloid. In hopes of doing better, drug companies are now working feverishly to develop novel antioxidants that cross into the brain easily and home in on β amyloid.

–ROBERT F. SERVICE

CREDIT: ALFRED PASIEKA, SCIENCE SOURCE/PHOTO RESEARCHERS